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## CORRECTIONS

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Page 190. The last line of Table V should read
"Checks 0/42 0/32 0.0".

Page 448. In line 7 "Whalley" should read "Whaley".

Pages 454 and 459. In lines 9 up and 11 up respectively, "Nitra" should read "Mitra".

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# THE EFFECT OF CERTAIN ELEMENTS, WITH EMPHASIS ON NITROGEN, ON THE PRODUCTION OF PERITHECIA OF VENTURIA INAEQUALIS (CKE.) WINT.[1]

R. G. Ross

## Abstract

Various elements were tested for their effect on the formation of perithecia of *Venturia inaequalis*. Apart from nitrogen the elements tested had little effect on the formation of perithecia except at relatively high concentrations. Perithecia did not form in media containing low concentrations of nitrogen and this was influenced by the source of nitrogen and by the cation present in nitrate salts. Perithecial initials developed in higher nitrogen concentrations than perithecia. Nitrogen added 9 weeks after mating of two lines of the fungus prevented the formation of perithecia.

Results were difficult to reproduce in a potato-dextrose apple leaf decoction medium but reproducible results were obtained in a synthetic medium.

## Introduction

The formation and production of fruiting bodies by fungi in artificial media often depends on the amount and nature of the available nutrients on which they are growing. In the formation of perithecia the ascomycetous fungi appear to be very sensitive to high concentrations of some nutrients. The formation of perithecia by *Valsa leucostoma* (Pers.) Fr. is checked by high concentrations of the sugars, maltose, sucrose, glucose, and levulose and by high concentrations of the nitrate salts of ammonium, calcium, sodium, and potassium (8). With *Melanospora destruens* Shear, Hawker (4) found that perithecial production decreased as the concentration of glucose was increased above 0.5% and was entirely inhibited at 2%. Keitt and Langford (7) were able to produce mature perithecia of *Venturia inaequalis* (Cke.) Wint. in agar containing 1.5% and lower concentrations of malt extract but not in that containing 2.5% malt extract. Westergaard and Mitchell (12) determined the influence of the carbon–nitrogen ratio on perithecial production by *Neurospora crassa* Shear and Dodge. High concentrations of glucose and potassium nitrate prevented formation of perithecia. Similar results were obtained with casein hydrolyzate and 0.5% ammonium tartrate. They attributed the effect of the latter to the ammonia ion. With the same fungus Hirsch (6) found that a high concentration of potassium nitrate inhibited the initiation of protoperithecia and that complete exhaustion of nitrate

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must occur before they develop into perithecia. Das Gupta and Nandi (3) tested the effect of various nitrogen concentrations on perithecial production in *Penicillium vermiculatum* Dangeard and found that too high a concentration of nitrogen checked production. Campbell (2) demonstrated a differential response to nitrogen in perithecial formation by two isolates of *Ceratocystis fimbriata* Ellis and Halst. Further examples of the effect of nutrition on perithecial formation are given by Lilly and Barnett (9) and Hawker (5).

Many fungi such as *Venturia inaequalis*, the causal organism of apple scab, reproduce sexually as saprophytes in overwintering leaves where undoubtedly the nutrient level falls as perithecial formation occurs. This change in nutrients presumably may effect the development of perithecia of *V. inaequalis*.

This paper reports the effect of some elements, with emphasis on nitrogen, that might be raised to excess in apple leaves, on the formation of perithecia of *V. inaequalis*.

## The Effect of Inorganic Compounds Containing Various Elements on Growth and Conidial Production of *V. inaequalis*

The effect of a number of elements contained in various inorganic compounds on the growth and asexual sporulation of *V. inaequalis* was determined as a preliminary to a study of their effect on perithecial formation. Dilution series of the compounds were added to test tubes containing Boone and Keitts' basal medium (1). This is a modified potato-dextrose agar supplemented with apple leaf decoction. The media and chemicals were autoclaved separately and then mixed. After cooling they were seeded with a suspension of either spores or spores and mycelium of *V. inaequalis*, agitated to achieve thorough mixing, and then slanted. Two tubes were prepared for each concentration of each chemical. After about two weeks' incubation at room temperature the tubes were examined and the amount of growth recorded by classification into four grades. Slides were prepared from each slant and examined under the microscope for sporulation.

The growth data are given in Table I. The compounds containing potassium, sodium, phosphorus, and molybdenum had no apparent effect on growth at the concentration used. The anion present had no effect on the toxicity of the copper and cobalt salts but in the compounds of the remaining elements, the effect of the anion on toxicity varied with the different cations.

Potassium, sodium, phosphorus, calcium, and molybdenum had no noticeable influence on conidial formation but high concentrations of the other elements decreased and in some instances almost completely inhibited sporulation. With magnesium, copper, zinc, and cobalt, conidial production declined as the concentration of the salts increased. The zinc salts almost completely inhibited sporulation, the conidia present were small and misshapen and, in a crushed mount, usually remained attached to the conidiophores. Conidia were very rare in those concentrations of manganese and iron just permitting growth of *V. inaequalis*. Low amounts of cobalt appeared to stimulate conidia production. No differences were observed between the different forms in which each element was tested.

TABLE I

Effect of various elements on the growth of *V. inaequalis*

| Element | Initial concn., p.p.m. | Compound | Growth rating* at various dilutions | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1/2 | 1/4 | 1/8 | 1/16 |
| K | 5000 | KCl | 3 | 3 | 3 | 3 | 3 |
| | | $KNO_3$ | 3 | 3 | 3 | 3 | 3 |
| | | $K_2SO_4$ | 3 | 3 | 3 | 3 | 3 |
| Na | 5000 | NaCl | 3 | 3 | 3 | 3 | 3 |
| | | $NaNO_3$ | 3 | 3 | 3 | 3 | 3 |
| | | $Na_2SO_4$ | 3 | 3 | 3 | 3 | 3 |
| Mn | 5000 | $MnCl_2$ | 0 | 0 | 2 | 3 | 3 |
| | | $Mn(NO_3)_2$ | 0 | 2 | 3 | 3 | 3 |
| | | $MnSO_4$ | 0 | 0 | 2 | 3 | 3 |
| Mg | 5000 | $MgCl_2$ | 2 | 2 | 3 | 3 | 3 |
| | | $Mg(NO_3)_2$ | 1 | 2 | 3 | 3 | 3 |
| | | $MgSO_4$ | 3 | 3 | 3 | 3 | 3 |
| P | 5000 | $NaH_2PO_4$ | 3 | 3 | 3 | 3 | 3 |
| | | $KH_2PO_4$ | 3 | 3 | 3 | 3 | 3 |
| Ca | 5000 | $CaCl_2$ | 2 | 2 | 2 | 2 | 2 |
| | | $Ca(NO_3)_2$ | 3 | 3 | 3 | 3 | 3 |
| Zn | 2500 | $ZnCl_2$ | 0 | 0 | 1 | 2 | 2 |
| | | $Zn(NO_3)_2$ | 0 | 0 | 1 | 2 | 2 |
| | | $ZnSO_4$ | 0 | 0 | 1 | 2 | 2 |
| Cu | 800 | $CuCl_2$ | 2 | 3 | 3 | 3 | 3 |
| | | $Cu(NO_3)_2$ | 2 | 3 | 3 | 3 | 3 |
| | | $CuSO_4$ | 2 | 3 | 3 | 3 | 3 |
| Fe | 300 | $FeCl_3$ | 0 | 1 | 2 | 3 | 3 |
| | | $Fe_2(SO_4)_3$ | 0 | 0 | 3 | 3 | 3 |
| Co | 150 | $CoCl_2$ | 1 | 3 | 3 | 3 | 3 |
| | | $Co(NO_3)_2$ | 1 | 3 | 3 | 3 | 3 |
| | | $CoSO_4$ | 1 | 3 | 3 | 3 | 3 |
| Mo | 50 | $(Na)_2MoO_4$ | 3 | 3 | 3 | 3 | 3 |
| | | $(NH_4)_6Mo_7O_{24}$ | 3 | 3 | 3 | 3 | 3 |
| | | $H_2MoO_4$ | 3 | 3 | 3 | 3 | 3 |

*0, no growth; 1, very little growth; 2, growth slightly less than checks; 3, same as checks.

## Experiments on Perithecial Formation in *V. inaequalis*

*General Methods*

The methods used to produce the perithecia of *V. inaequalis* in vitro were similar to those described by Keitt and Langford (7). The basal medium used was the same as that employed in the preliminary growth studies. Two sexually compatible wild-type lines of the fungus, 365-4 and 1096, supplied by Dr. D. M. Boone of the University of Wisconsin, Madison, Wisconsin, were used throughout and paired for seeding the basal medium containing the chemicals under test. As in the growth experiments a series of concentrations of each chemical prepared in the basal medium was used, the maximum concentration being one permitting growth of the fungus. Three 4-cm Petri dishes poured with 6 to 7 ml of the amended medium were used for each concentration of each chemical. The inoculum consisted of a mixture of

365-4 and 1096 prepared by macerating an agar slant of each isolate in sterile water in a Waring blendor. Controls consisted of pairings in unamended basal medium. The cultures were incubated at room temperature for 2 weeks and then at 8° C for 4 to 5 months, at which time notes were taken on perithecial formation.

*The Effect of Various Cations as Chloride, Sulphate, and Nitrate Salts on the Development of Perithecial Initials and the Maturation of Perithecia*

The results presented in Table II show the effects of various cations, tested as chloride, sulphate, or nitrate salts, on the formation of perithecial initials and the maturation of perithecia. As would be expected, the inhibiting levels of various cations varies greatly. Further, it is apparent from the results that *V. inaequalis* can complete its sexual cycle in the presence of much higher concentrations of chlorides and sulphates than nitrates. Where inhibition of perithecia occurred, the compounds were more toxic to the formation of perithecia themselves than to the formation of initials, but under these circumstances the initials were usually very small. At concentrations just permitting formation of perithecia, these were usually immature and contained very few ascospores, but as the concentration decreased, normal mature perithecia developed. In some instances there appeared to be a stimulation of perithecial production.

TABLE II

Maximum concentration of cations in different salts permitting formation of initials and perithecia

| Cation | Initials | | | Perithecia | | |
|---|---|---|---|---|---|---|
| | $Cl^-$ | $SO_4^=$ | $NO_3^-$ | $Cl^-$ | $SO_4^=$ | $NO_3^-$ |
| $K^+$ | >5000* | >5000 | 1250 | 2500 | 2500 | <625 |
| $Na^+$ | 2500 | 2500 | 1250 | 1250 | 1250 | <625 |
| $Ca^{++}$ | >5000 | | 1250 | 2500 | | <625 |
| $Mg^{++}$ | 1250 | 1250 | 625 | 625 | 625 | <625 |
| $Mn^{++}$ | >1000 | >1000 | >1000 | >1000 | >1000 | 125 |
| $Zn^{++}$ | >500 | >500 | 250 | 250 | 250 | <125 |
| $Cu^{++}$ | 125 | 125 | 125 | 62 | 125 | 125 |
| $Fe^{+++}$ | >100 | >100 | | >100 | >100 | |
| $Co^{++}$ | >50 | >50 | >50 | >50 | >50 | 25 |

*Concentration of cation in p.p.m. Where positive values are given the next higher concentration was double the one shown here.

In addition to the compounds represented in Table II certain phosphates and molybdates were also examined. These had no appreciable effect on perithecial production.

*Effect of Different Nitrate Salts on Perithecial Formation*

The results reported in the previous section indicated that, with nitrate salts, the concentration of nitrogen in which perithecia formed varied with the cation present. An experiment was then carried out in which different nitrate salts were added to the basal medium in amounts calculated to give equivalent concentrations of nitrogen. The data in Table III show the maximum concentrations of nitrogen in which initials and perithecia developed. Perithecia developed in 250 p.p.m. nitrogen supplied as potassium nitrate

but were inhibited at much lower concentrations with other nitrate salts. The results would indicate that the differences in toxicity between the different salts were due to an additive effect of the cation and anion. The action of copper was different than the other metals, the same concentration inhibiting both initials and perithecia.

TABLE III

Maximum concentration of nitrate nitrogen with different cations permitting formation of initials and perithecia

| Cation | Initials | Perithecia |
|---|---|---|
| $K^+$ | >500* | 250 |
| $Mg^{++}$ | >500 | 125 |
| $Mn^{++}$ | 250 | 62 |
| $Zn^{++}$ | 50 | 25 |
| $Cu^{++}$ | 12 | 12 |
| $Co^{++}$ | >25 | <3 |

*Concentration of nitrate nitrogen in p.p.m. Where positive values are given the next higher concentration was double the one shown here.

*Effect of Different Forms of Nitrogen on Perithecial Formation*

To determine the effect of nitrogen from various sources on the development of initials and perithecia the nitrogen compounds in Table IV were used. They were first tested at nitrogen concentrations ranging from 500 to 62.5 p.p.m. Perithecia developed normally in the basal medium but did not develop in any of the nitrogen treatments. Some initials were present at the lower concentrations. The experiment was repeated in a series of dilutions beginning at 100 p.p.m. nitrogen. In this experiment perithecia developed at all nitrogen concentrations and were somewhat more abundant in the checks than in the previous trial. The experiment was then carried out in a series of concentrations beginning at 250 p.p.m. nitrogen; the results are shown in Table IV. In potassium nitrate, ammonium nitrate, and ammonium sulphate at 250 p.p.m. nitrogen, the perithecia were immature and contained very few asci and ascospores, which indicated that these salts were very near their maximum concentration for perithecial formation. There were no apparent differences between the three salts. Nitrogen supplied as amino acids was somewhat more toxic but there was little difference between asparagine and glycine. Sodium nitrate was slightly more toxic than the potassium salt.

TABLE IV

Maximum concentration of nitrogen from different sources permitting formation of initials and perithecia

| Source | Initials | Perithecia |
|---|---|---|
| $KNO_3$ | >250* | >250 |
| $NaNO_3$ | >250 | 200 |
| Asparagine | 150 | 100 |
| Glycine | 100 | 100 |
| $NH_4NO_3$ | >250 | >250 |
| $(HN_4)_2SO_4$ | >250 | >250 |

*Concentration of nitrogen in p.p.m. Where positive values are given the next higher concentration was 50 p.p.m. more than the one shown here.

*Effect of Nitrogen Added at Various Intervals during Perithecial Formation*

Preceding experiments have shown that the initial concentration of nitrogen in the media is a limiting factor in the formation of perithecia. These experiments, however, provide no information on the effect of nitrogen at different times during the development of perithecia. Consequently, studies were done on the effect of adding nitrogen at various times during perithecial development.

In this experiment basal medium containing inoculum was prepared at 1.25× and dispensed in 60-mm disposable plastic Petri dishes, 13.33 ml per dish. Immediately, and every 3 weeks for 18 weeks, a separate series of 12 of these plates were treated as follows: four each received 3.33 ml of a sterile solution of 10,000 p.p.m. nitrogen as potassium nitrate, four received a similar solution containing 5000 p.p.m. nitrogen, and the other four received sterile water. The nitrogen-treated plates thus contained final nitrogen concentrations of 2000 and 1000 p.p.m.

Perithecia developed in all plates where water had been added. The results of those receiving the nitrogen solutions are presented in Table V. The interval after mating at which additions of nitrogen adversely affected the formation of initials and maturation of perithecia depended on the concentration added. Apparently after a certain stage in the development of perithecia, nitrogen does not further affect their maturation.

TABLE V

Effect of nitrogen added at various times during development of perithecia

| No. of weeks from mating to addition of nitrogen | Perithecia and initials at two concentrations of nitrogen supplied as potassium nitrate | | | |
|---|---|---|---|---|
| | 2000 p.p.m. | | 1000 p.p.m. | |
| | I | P | I | P |
| 0 | − | − | − | − |
| 3 | − | − | + | − |
| 6 | + | − | + | − |
| 9 | + | − | + | + |
| 12 | + | + | + | + |

*Production of Perithecia on Synthetic Media*

Previous experiments have shown the difficulty in reproducing results using the potato-dextrose apple leaf decoction medium, which contains plant extracts and thus is difficult to standardize for each experiment. Consequently, experiments were carried out on the production of perithecia of *V. inaequalis* using synthetic media.

The basal synthetic medium was modified from the one used by Pelletier and Keitt (10). A liter contained: $KH_2PO_4$, 1.5 g; $MgSO_4.7H_2O$, 0.5 g; glucose (anhydrous), 5.0 g; Tween 80, 1.0 g; trace elements (μg/l.: B, 5; Mn, 10; Cu, 20; Zn, 90; Fe, 100; Co, 100); vitamins (μg/l.: thiamine, 250; inositol, 1000; pantothenic acid, 500; nicotinic acid, 500; pyridoxin, 125); Difco Special Agar (Noble), 17.0 g. Glass distilled water was used throughout and all glassware was cleaned with chromic–sulphuric acid solution with a final rinse in distilled water. Inoculum for seeding was obtained from wick cultures

described by Keitt and Langford (7). Spores were harvested from 14- to 18-day-old wicks grown at 18° C and washed three times before seeding. Difco Bacto-Casamino acids or potassium nitrate was added to the basal media to give a series of nitrogen concentrations ranging from 25 to 400 p.p.m.

Before testing for perithecial development the *V. inaequalis* isolates 1096 and 365-4 were seeded separately on these media and examined for growth after 2 weeks. Both isolates grew on all media but the growth of 1096 was somewhat better than that of 365-4. On both nitrogen sources the amount of growth increased as the concentration of nitrogen increased.

The results on development of perithecia are given in Table VI. The test was done at three different times with almost identical results. The cutoff points for perithecia and initials were the same, but there was some variation in the relative number of perithecia produced at different concentrations of nitrogen. Nitrogen supplied as potassium nitrate was more toxic to the maturation of perithecia than that supplied by casamino acid but the latter was more toxic to the formation of initials. With casamino acids the cutoff point for initials and perithecia was the same. This has not occurred with any of the other nitrogen compounds tested. It is interesting to note that with potassium nitrate, at 100, 200, and 300 p.p.m. nitrogen, the initials were very small; thus the basal medium may have had some effect on these structures.

TABLE VI

Maximum concentration of nitrogen in synthetic media permitting formation of initials and perithecia

| Source | Initials | Perithecia |
|---|---|---|
| Casamino acids | 100* | 100 |
| $KNO_3$ | 300 | 50 |

*Concentration of nitrogen in p.p.m.

## Discussion

The effect of nitrogen on perithecial formation in *V. inaequalis* follows that found in other fungi (5, 6, 8, 9, 12). Among the compounds tested in this work, only those containing nitrogen had a significant harmful effect on perithecial formation but other workers (7) have shown that perithecia of *V. inaequalis* do not develop in high concentrations of malt extract. Sugars would probably have a similar effect since they inhibit perithecial development in many fungi (4, 5, 8, 9).

The concentration of nitrogen in which perithecial initials formed and developed into mature perithecia depended on the source of nitrogen and the cation present in nitrate salts (Tables III and IV). The latter was probably due to differences in the toxicity of cations. Perithecial initials formed in nitrogen concentrations that prevented their ensuing development into perithecia. After a certain stage in the formation of perithecia additions of nitrogen had no apparent effect (Table V). With *Neurospora crassa*, Hirsch (6) found that complete exhaustion of nitrate must occur before protoperithecia develop into mature perithecia while Leonian (8) concluded that an absence or decrease of food was not a factor in perithecial formation of *Valsa*

*leucostoma*. In these experiments with *V. inaequalis* it seems unlikely that the organism could utilize or exhaust the large amount of nitrogen added at 12 weeks (Table V). Thus it appears that perithecia of *V. inaequalis* are able to mature in the presence of nitrate nitrogen, the nitrogen effect occurring at an earlier stage in the sexual cycle.

The production of perithecia on synthetic media affords an opportunity to study more precisely, with reproducible results, the effect of nutrition on development of perithecia of *V. inaequalis*. Perithecia were more sensitive to potassium nitrate in the synthetic than in the potato-dextrose apple leaf decoction medium. Robbins (11) found that the addition of certain plant extracts reduced the inhibiting effect of asparagine on zygospore formation in *Phycomyces blakesleeanus* Burgeff.

In nature, *V. inaequalis* produces perithecia in the overwintered apple leaves. Throughout the fall and winter these leaves are subjected to decay and leaching, which undoubtedly changes and lowers the nutrient level. In the development of perithecia in artificial media, *V. inaequalis* is very sensitive to low concentrations of some nutrients. In nature a change in the nutrient level of the apple leaves may be necessary for perithecial development to occur. This would seem to have some practical implications in the control of apple scab. In most apple-growing areas the organism overwinters by the formation of perithecia in fallen apple leaves. It may be possible to modify the nutrition of this environment and prevent the development of perithecia. This possibility is being investigated.

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# MECHANISMS CONTRIBUTING TO THE ERADICATION OF SPORES OF HELMINTHOSPORIUM SATIVUM FROM AMENDED SOIL[1]

S. H. F. CHINN AND R. J. LEDINGHAM

## Abstract

Declines in population of spores of *Helminthosporium sativum* in amended soil were due to disappearance that occurred in normal soil and to eradication brought about by the germination–lysis mechanism and by an increase in antagonism. Germination–lysis played a major role in molasses- and vitamin C-amended soils but not in glucose-amended soil, as was evidenced by the density of spore populations in the soils at intervals over a 16-week period.

Antagonism which was increased in all three amended soils was responsible to a lesser extent for the eradication of the spores. The data suggest that antagonism was more effective during the early, than during the later, stages in the breakdown of the amendment. A close correlation was noted between spore population and the incidence of seedling infection. Correlation between spore population and disease ratings was much less significant.

## Introduction

According to a preliminary study by Chinn *et al.* (4), common root rot of wheat can be controlled by the addition of soybean meal to the infested soil. They demonstrated that the amendment promoted germination of the spores of *Helminthosporium sativum* Pamm., King, and Bakke (*Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur), the main causative organism of the disease. Subsequently, lysis of the germ tubes occurred, thus suggesting the mechanism responsible for the eradication of the pathogen. They (unpublished data) also noted a very substantial increase in the soil flora as a result of the amendment, and surmised that the enhanced microbial activity might be associated with the formation of substances that inhibited or destroyed the pathogen. This mechanism, referred to as antagonism by numerous authors (e.g., 5, 9, and 14), is the commonly accepted explanation for the biological control of various soil-borne plant diseases.

This paper reports the results of further study on the role of the germination–lysis mechanism in the eradication of *H. sativum* spores from soil by various amendments. Since antagonism occurred simultaneously in all the amended soils, this study was expanded to include an assessment of the importance of the two mechanisms in the biological control of common root rot, with special reference to *H. sativum*. The relationships between spore population, disease ratings, and seedling infections were also determined.

## Materials and Methods

### *Substances Used as Amendments*

Glucose, molasses, and vitamin C were used to amend the soil. These substances were selected because of their contrasting capacities to promote

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Contribution No. 80, Canada Department of Agriculture Research Station, Saskatoon, Saskatchewan.

germination of spores of *H. sativum* in soil (2). The rates of amendment were 1.25% for glucose and molasses and 1% for vitamin C. Preliminary tests indicated that for molasses and vitamin C these were about the minimum rates that would promote 100% germination of the spores. Glucose was added at the same rate as molasses with the thought that there would not be too large a difference in the microbial activities in the two amended soils.

*Soil*

A medium loam soil practically devoid of spores of *H. sativum* was screened, and water was added to bring it to 50% of its moisture-holding capacity. The soil was divided into 12 lots, 4 weighing 1 kg each and 8 weighing 10 kg each. Some lots received treatment immediately; others were kept for a later date.

*Inoculum*

A strain of *H. sativum* (H5) was cultured on sterile, moist straw in test tubes and stored for about two months, by which time the straw was dehydrated. A water suspension of the spores was used in the various experiments and determinations.

*Determinations of Germination–Lysis and Population of Spores of H. sativum in Soil*

The influence of amendments on the germination and lysis of spores of *H. sativum* in soil was determined by the slide technique (1). According to this technique, spores are embedded in plain agar on slides and introduced into the soil. In this study, spores were introduced at specified times. The slides remained in the soil for 16 to 20 hours before they were removed and examined.

The population of spores of *H. sativum* in soil was determined by the flotation–viability count method (3). Although total counts were taken, only the viable spores are reported. The spore population was recorded as number per slide rather than number per gram of soil.

## Experimental Results

*Effect of Time of Introduction of Spores on Their Germination in Amended Soil*

The effect of time of introduction of spores on germination and lysis was studied in glucose-, molasses-, and vitamin C-amended soils. Three of the 1-kg lots of soil were amended singly with the above substances and the fourth lot was kept as a control. Determinations of spore germination and lysis were made at the time of, and 7 days after, the amendment.

TABLE I

Percentage germination of *H. sativum* spores introduced into soil amended with glucose, molasses, or vitamin C

| Time of introduction of spores to the soil | Amendment | | | |
|---|---|---|---|---|
| | Glucose | Molasses | Vitamin C | Nil (control) |
| At time of amendment | 2 | 94 | 98 | 0 |
| 7 days after amendment | 0 | 0 | 2 | 0 |

The results of the determinations are shown in Table I. Nearly 100% of the spores germinated in the molasses- and vitamin C-amended soils where the spores were introduced at time of amendment. With a minor exception, no germination occurred in these soils when the spores were added 7 days after the amendment. Germination was practically nil in the glucose-amended soil regardless of whether the spores were introduced at the time of, or 7 days after, the amendment. There was no germination in the non-amended soil.

Lysis of germ tubes was observed usually 4 to 5 days after germination.

*Effect of Time of Addition of Spores on Their Eradication in Amended Soil*

The effect of time of addition of the spores on their eradication was tested in the 10-kg lots of soil. As shown in Table II, lots 4, 5, and 6 were amended with glucose, molasses, and vitamin C, respectively. After treatment, the soil was placed in crocks, covered with foil, and stored at room temperature. Seven days later, lots 1, 2, and 3 were similarly amended and in the same order. Aliquots of a spore suspension of *H. sativum* of predetermined spore density were added to all six lots of amended soil, and as well to lot 7, which was not amended. Lot 8 served as a control. Thus spores in lots 4, 5, and 6 were added 7 days after treatment and those in lots 1, 2, and 3 at the time of amendment. All soil lots were stored as mentioned above, and sterile water was added periodically to maintain the original moisture level during the experiment. Soil prepared for this experiment was also used in subsequent experiments reported in this study.

The spore population of all eight lots of soil was determined at 0, 1, 2, 4, 8, 12, and 16 weeks after the spores were added to the soil. Spore counts at the different dates over the 16 weeks are shown in Table II. The spore population of the seven lots of infested soil at 0 week ranged from 17 to 25 per slide, suggesting a fair degree of uniformity in the addition of inoculum. Only one spore was observed in the seven determinations made on the control soil.

In the non-amended and infested soil (lot 7) the population declined gradually to less than half in 16 weeks. In the molasses- and vitamin C-amended soils where the inoculum was added at time of amendments (lots 2 and 3) spores declined in number substantially by the end of 1 week, and had practically disappeared by the end of 2 weeks. In the remaining four lots (1, 4, 5, and 6) the declines were less than in the above two lots, and the populations still ranged from 3 to 11 spores per slide at 16 weeks. However, with the exception of a determination made on the 16th week in lot 5, the declines were greater than in the non-amended soil (lot 7).

*Effect of Time of Addition of Spores on Infection of Wheat Seedlings in Amended Soil*

The effect of time of addition of spores on infection was determined by noting the lesions of wheat seedlings grown in amended soil. Soil prepared for the previous experiment was used. Each lot was seeded at 1, 4, 8, 12, and 16 weeks after the addition of spores. Twenty-five wheat seeds were sown in each $\frac{1}{4}$-gal crock and there were four replicates. The seedlings were harvested 21 days after seeding, and disease ratings, based on severity of the lesions, were made according to a method described by Ledingham, Sallans, and Wenhardt (10).

TABLE II

Spore counts obtained at various intervals over a 16-week period in amended soil to which spores of *H. sativum* were added at the time of, and 7 days after, amendment

| Lot No. | Amendment | Spores added | Spores per slide | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 4 (weeks) | 8 | 12 | 16 | Total* |
| 1 | Glucose | At time of amendment | 25 | 20 | 22 | 9 | 4 | 3 | 3 | 39 |
| 2 | Molasses | At time of amendment | 21 | 12 | 0 | 0 | 1 | 0 | 0 | 13 |
| 3 | Vitamin C | At time of amendment | 20 | 4 | 1 | 0 | 1 | 1 | 0 | 6 |
| 4 | Glucose | 7 days after amendment | 22 | 24 | 18 | 12 | 6 | 5 | 6 | 53 |
| 5 | Molasses | 7 days after amendment | 17 | 22 | 19 | 11 | 10 | 5 | 11 | 59 |
| 6 | Vitamin C | 7 days after amendment | 18 | 10 | 4 | 9 | 3 | 6 | 4 | 32 |
| 7 | Nil | Same time as lot 1 | 24 | 21 | 18 | 27 | 15 | 10 | 9 | 82 |
| 8 | Nil (control) | Nil | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |

*Totals of counts made at 1, 4, 8, 12, and 16 weeks were used for correlation study.

TABLE III

Disease ratings and incidence of infection of wheat seedlings grown at various intervals over a 16-week period in amended soil in which spores of *H. sativum* were added at the time of, and 7 days after, amendment

| Lot No. | Amendment | Spores added | Disease ratings when seeded after (weeks) | | | | | | Incidence of *H. sativum** (weeks) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 4 | 8 | 12 | 16 | Total† | 1 | 4 | 8 | 12 | 16 | Total† |
| 1 | Glucose | At time of amendment | 7 | 10 | 4 | 21 | 31 | 73 | 7 | 13 | 1 | 4 | 13 | 38 |
| 2 | Molasses | At time of amendment | 5 | 6 | 4 | 8 | 19 | 42 | 2 | 5 | 0 | 1 | 2 | 10 |
| 3 | Vitamin C | At time of amendment | 10 | 5 | 4 | 16 | 11 | 46 | 2 | 1 | 0 | 1 | 2 | 6 |
| 4 | Glucose | 7 days after amendment | 7 | 9 | 6 | 7 | 15 | 44 | 5 | 14 | 2 | 6 | 11 | 38 |
| 5 | Molasses | 7 days after amendment | 6 | 9 | 5 | 11 | 19 | 50 | 13 | 16 | 8 | 12 | 16 | 65 |
| 6 | Vitamin C | 7 days after amendment | 6 | 4 | 2 | 12 | 10 | 34 | 0 | 3 | 2 | 6 | 13 | 24 |
| 7 | Nil | Same time as lot 1 | 14 | 20 | 7 | 19 | 21 | 81 | 21 | 22 | 15 | 17 | 19 | 94 |
| 8 | Nil (control) | Nil | 3 | 3 | 1 | 11 | 4 | 22 | 0 | 2 | 0 | 0 | 0 | 2 |

*Incidence of infection by *H. sativum* of 24 most-lesioned seedlings.
†Totals of disease ratings and incidence of *H. sativum* were used for correlation study.

The incidence of infection by *H. sativum* was determined by plating the basal portion of 24 of the most severely lesioned seedlings from each treatment on acidified potato-dextrose agar. Infection caused by *H. sativum* was recorded and some attention was given to infection caused by fungi other than *H. sativum*.

The results of the experiment are shown in Table III. With one exception the lowest disease ratings and least number of *H. sativum* isolates were obtained from seedlings grown in the control soil (lot 8). The exception, a disease rating of 11, was noted in the planting made 12 weeks after the start of the experiment. In this case, none of the infections was caused by *H. sativum*; rather 10 of the culms were infected by *Fusarium acuminatum*. With two exceptions, the highest disease ratings and the largest number of *H. sativum* isolates occurred among the seedlings grown in the non-amended infested soil (lot 7) regardless of seeding date.

Among the different amendments, disease ratings, with a few exceptions, were quite uniform within any one seeding date. Practically all the disease ratings of the seedlings from each amended soil were lower in the first three seedings than in the last two seedings. The numbers of *H. sativum* isolates were much lower from the seedlings grown in the molasses- and vitamin C-amended soils into which the spores were added at time of amendment (lots 2 and 3) than from those grown in similarly amended soil in which the spores were added 7 days after the amendment (lots 5 and 6), or those grown in either of the glucose-amended soils (lots 1 and 4).

*Effect of Amendment on Soil Flora and Soil Reaction*

The effects of glucose, molasses, and vitamin C amendments on the quantitative changes in the bacterial, actinomycetous, and fungal flora and on changes in pH was studied on four lots of soil from the previous experiments. These lots included one of each of the above amendments and a control. Estimation of numbers was made by the soil dilution plate technique using soil extract agar, Conn's medium, and peptone dextrose agar (7) for the plating of bacteria, actinomycetes, and fungi, respectively. Soil reaction was determined with a Beckman potentiometer. All determinations were made 0, 1, 4, 8, 12, and 16 weeks after the amendment.

The changes in numbers of bacteria and fungi are shown in Figs. 1 and 2. Glucose and molasses increased the bacterial population substantially, whereas vitamin C had little influence. Maximum counts occurred sooner in the glucose-amended than in the molasses-amended soils; the bacterial counts in both soils remained fairly high at 16 weeks. All three substances increased the fungal population; the influence of vitamin C was particularly marked. The populations of the fungi in the three amended soils declined by the 16th week, but they were still considerably higher than in the control soil.

Glucose and molasses increased the population of actinomycetes similarly, but less extensively than the bacteria. However, vitamin C, due to its acidic nature, appeared to decrease the population of actinomycetes (it was difficult to estimate accurately the numbers of actinomycetes because of the rapid growth of fungi on the plates).

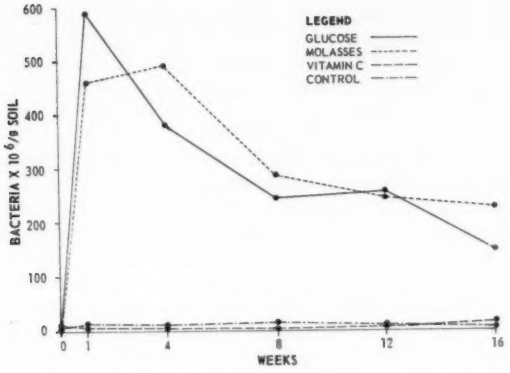


FIG. 1. Changes in numbers of bacteria over a period of 16 weeks in soil amended with glucose, molasses, or vitamin C.

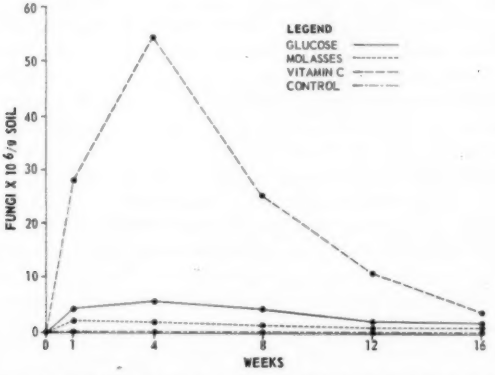


FIG. 2. Changes in numbers of fungi over a period of 16 weeks in soil amended with glucose, molasses, or vitamin C.

The pH of the control soil ranged from 5.9 to 6.1 in the determinations made during the 16 weeks. Glucose and molasses influenced only slightly the pH of the soil at the beginning of the amendment. The pH of both of these amended soils increased to nearly 7 in 1 week and then decreased to about 6.4 for the rest of the experiment. Vitamin C lowered the pH of the soil to 4.9 initially. After 1 week the pH was 5.6 and continued to increase to 6.6 in 16 weeks.

*Comparison of Spore Population with Disease Ratings and Numbers of Seedlings Infected by H. sativum*

The totals of spore counts, disease ratings, and numbers of seedlings infected by *H. sativum* made at 1, 4, 8, 12, and 16 weeks were used for a correlation

study (Tables II and III). A correlation coefficient of 0.714 was found between spore populations in the soil and disease ratings of wheat seedlings grown in the soil in the greenhouse. A much higher coefficient, 0.967, was found between spore population and number of seedlings infected by *H. sativum*.

## Discussion

Antagonism has been perhaps the most recognized explanation for the biological control of soil-borne plant pathogens. The data obtained in this study indicated, however, that another mechanism may play an even greater role in the eradication of spores of *H. sativum* from amended soil. This mechanism, namely germination–lysis, has been postulated earlier (4), but it is only now, as a result of the development of a newer technique (3), that the postulation has been confirmed. This concept is analogous to the findings of Mitchell, Hooton, and Clark (11), who attributed germination as the cause of the disappearance of sclerotia of *Phymatotrichum omnivorum* in soil amended with organic matter. The evidence presented here suggests that, in future investigations concerning the eradication of fungal spores from soil, some thought should be given to both mechanisms.

The study indicates that in amended soil the mechanisms of germination–lysis and antagonism played major roles in the eradication of spores of *H. sativum*. However, other forces also contributed to the declines in the population of the pathogen; a certain percentage of spores simply disappeared and this was attributed to physiological aging or other factors occurring under natural conditions. In the non-amended soil (lot 7, Table II) about 50% of the spores were still viable at the end of 16 weeks. This indicates the survival rate or, conversely, the disappearance rate of the *H. sativum* under the conditions that were considered natural in the experiment.

In the amended soil, spore eradication was caused by one or both of the suggested mechanisms. The data in Table I indicate that molasses and vitamin C promoted germination of spores of *H. sativum* followed by lysis of the germ tubes. Glucose, however, was unable to produce these effects. Thus, eradication of spores would be expected in either the molasses- or vitamin C-, but not in the glucose-amended, soil. With molasses and vitamin C amendments (lots 2 and 3, Table II) the spore population declined greatly in 1 week and almost completely in 2 weeks, whereas with glucose amendment (lot 1, Table II) the decline was gradual.

Additional confirmation suggesting the germination–lysis mechanism for spore eradication was also obtained. Other data in Table I show that molasses and vitamin C promoted germination of those spores that were in the soil at the time of amendment but not those introduced 1 week later. Under the latter condition any decline of spore population could not be attributed to the germination–lysis mechanism. Data in Table II show that with the molasses- and vitamin C-amended soils, where the spores were added 1 week after amendment (lots 5 and 6), the population declined gradually, and to a lesser extent than with similarly amended soil (lots 2 and 3) where the spores were added at time of amendment. The decline was more or less comparable to that noted in either of the glucose-amended soils (lots 1 and 4) where germination did not occur and, consequently, could not be a factor in spore eradication.

The results also suggest that antagonism was partly responsible for the eradication of spores of *H. sativum* in the amended soil. In the four lots of amended soil (lots 1, 4, 5, and 6, Table II) where the germination–lysis mechanism was not applicable because spore germination was not evident, the decline in population was more pronounced than that of the non-amended soil. The difference could be attributed to the antagonistic effect brought about by the increased microbial activity in the soil. It is not possible with the evidence available to indicate which organisms or groups of organisms were responsible for the production of antibiotics fungicidal to *H. sativum*. Possibly, the fungal group was responsible as it was the only one influenced greatly in the vitamin C-amended soil. Nevertheless, bacteria and actinomycetes have been suggested by various authors (e.g., 5, 6, 8, and 13) as contributing to antagonistic effects against *H. sativum* and other pathogens in soil. These two groups of organisms increased significantly in molasses- and glucose-amended soils.

Another aspect of antagonism is suggested. The disease ratings of seedlings grown in the four lots of amended soil (lots 1, 4, 5, and 6, Table III), where the germination–lysis mechanism was not involved, indicate that the amendment was more effective in the earlier dates of seeding (first three) than the later dates of seeding (last two). The effectiveness coincided with maximum microbial activities. This is in general agreement with observations made by Papavizas and Davey (12), who suggested that easily decomposable material gave best control of *Rhizoctonia* 1 to 3 weeks after incorporation. It is quite probable that during the earlier dates of decomposition, fungistatic substances were formed in quantity, resulting in the inhibition of the pathogen. Subsequently, this effectiveness decreased, possibly because of the relatively slower formation but faster breakdown of these substances.

That the number of spores of *H. sativum* in soil is not closely related to common root rot of wheat is indicated by a correlation coefficient of 0.714 between spore population and disease ratings. This suggests probably the influence of environmental conditions on the capacity of the pathogen to infect the plant or the presence of other fungi capable of causing similar symptoms. One striking example was the isolation of a greater number of *Fusarium acuminatum* than of *H. sativum* from infected seedlings harvested from the 8-week seeding. This *Fusarium* was isolated only occasionally from seedlings at the other periods.

On the other hand, excellent correlation (0.967) existed between spore population and the number of seedlings infected by *H. sativum*. More experiments, however, will be required to establish definitely this relationship.

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# NUCLEAR STRUCTURE AND DIVISION IN THE MYCELIUM AND BASIDIOSPORES OF CERATOBASIDIUM PRATICOLUM[1]

H. K. SAKSENA[2]

## Abstract

Nuclear structure and division in the mycelium and basidiospores of *Ceratobasidium praticolum* (*Pellicularia praticola*), studied in living and in differently stained preparations, are described and illustrated with photographs. The cells of the mycelium are multinucleate. Nuclear migration occurs from one cell to another through the septal pores. The nuclei consist of a Feulgen-negative nucleolus surrounded by granular Feulgen-positive chromatin. In a nucleus preparing to divide, the nucleolus becomes progressively smaller and finally disappears. The chromatin then elongates, becoming bar-like, and later constricts at the mid-region. New nucleoli appear in the two attached portions of the constricted chromatin. At the end of the division the two portions pull apart to form two sister nuclei. Classic mitotic division as it occurs in higher organisms was not seen. Chromosomal filaments were not discerned in the nuclei of the basidiospores or the mycelium. Chromosomal filaments in reticulate arrangement were observed in the nuclei of the hymenial cells and in the cells of the short, side branches on which the hymenium develops.

## Introduction

*Ceratobasidium praticolum* (Kotila) Olive (*Pellicularia praticola* (Kotila) Flentje) causes damping-off in seedlings of agricultural, horticultural, and forest plants in various parts of the world. The results of investigation on the morphology and pathogenicity, and on the cytology of the basidium, of *C. praticolum* have been recently reported from this Laboratory (15, 16). The present paper deals with the structure and mode of division of the nuclei in the vegetative mycelium and basidiospores of this species.

The numerous cytological studies made in the past on fungi deal mainly with the behavior of nuclei in the sexual cells during meiosis (12). The stages in the meiotic division in general proceed very much as in the higher plants. The behavior of the nuclei in the vegetative cells of fungi has recieved little attention till recently, and the vegetative nuclei reportedly differ in their mode of division. Among the recent workers on the cytology of fungi, Knox-Davies and Dickson (11) consider that the vegetative nuclei of *Helminthosporium turcicum* Pass. divide by ordinary mitosis. Cole and Couch (9) also report observing various mitotic stages during division of nuclei in the conidia and mycelial cells of *Kabatiella caulivora* (Kirch.) Karak. in living and in stained preparations. On the other hand, Widra (18) found that nuclear division in *Candida* spp. differed from classic mitotic division. According to the recent, well-illustrated accounts of Robinow (13, 14) and Bakerspigel (2, 3, 4, 5, 6, 7, 8), the vegetative nuclei in the fungi of different groups studied by them divide without the aid of a mitotic apparatus, such as a spindle or metaphase

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plate. The observations of Robinow and Bakerspigel are not in agreement with the prevalent belief that the nuclear divisions occurring in the vegetative phase of fungi are essentially the same as in higher organisms. Their cytological studies have laid a good basis for continuing and extending these investigations to other fungi.

## Materials and Methods

*Culture*

Isolate 1250 of *C. praticolum*, obtained from seedlings of *Pinus banksiana* Lamb., was studied previously (15, 16), and was used in these investigations. This isolate was maintained on 2% potato-dextrose agar (PDA). Basidiospore development was obtained on 2% corn-meal agar in the manner outlined formerly (16).

*Cytological Preparations*

*1. Mycelium*

Small (1.5 cm square) sterile strips of dialyzing cellophane, placed on the surface of PDA plates, were inoculated with 0.2-mm pieces of inoculum taken from 5-day-old cultures of *C. praticolum*, or by spreading a loopful of basidiospore suspension on the surface of the squares. The plates were incubated at 24° C until the desired growth was obtained.

The cytology of the mycelium grown on cellophane strips was studied mainly in Haidenhain's iron alum haematoxylin and HCl–Giemsa preparations. As will be seen later, these techniques complemented each other. Recent cytological studies of fungal mycelium have shown that Giemsa-stained nuclei compare well with nuclei stained in Feulgen (2, 3, 4, 5, 6, 7, 8, 13, 14). A number of Feulgen preparations were also studied to reveal the site of DNA in these nuclei, and to confirm the observations made in the HCl–Giemsa preparations.

The techniques used in fixing and staining the vegetative nuclei were essentially the same as those described in detail by Robinow (13) and Bakerspigel (2). The cellophane cultures to be stained with Giemsa or in Feulgen were fixed in a 3 : 1 mixture of absolute ethyl alcohol and glacial acetic acid, hydrolyzed in $\pm N$ hydrochloric acid for 10 minutes, and then stained in Giemsa for 1 hour, or in Feulgen for 3 hours. The Giemsa stain was prepared by adding 2 drops of Giemsa solution (Gurr's Improved Giemsa R-66) per ml buffer solution, pH 6.8. Cultures to be stained with iron alum haematoxylin were fixed in the vapor of 2% osmium tetroxide for 5 minutes, and then stained for 24 hours. Haematoxylin was used in the half-oxidized form as recommended by Baker and Jordan (1).

*2. Basidiospores*

The cytology of the basidiospores was studied in haematoxylin and HCl–Giemsa preparations as outlined in an earlier paper (15).

*Preparations for Phase-Contrast Microscopy*

Living nuclei in basidiospores, germ tubes, and cells of growing hyphae were studied with a phase-contrast microscope. Thin (single dip) films of PDA on slides, prepared as described by Robinow (13), were inoculated with

basidiospores by spreading a loopful of spore suspension. The preparation was examined soon after, or placed in a moist chamber for further growth. For examination, a sterile cover slip was placed over the seeded agar film and ringed with paraffin. For the study of hyphal nuclei, cellophane cultures were also examined on a slide in a few drops of sterile 1% glucose solution in water. A cover slip was then placed over the solution and sealed with paraffin.

*Photomicrography*

The photographs were taken on Kodak High Contrast Copy film using a Zeiss microscope fitted with a photographic apparatus described earlier (15). For phase microscopy, a Zeiss phase-contrast condenser, and phase apochromatic objective (N. A. 1.32), were used.

## Results

### I. The Living Nucleus

The living nuclei observed in the basidiospores, germ tubes, and hyphal cells are most commonly spherical or oval. The interdivisional nucleus (Figs. 1–5) consists of an optically dense central body, hereafter called the nucleolus, surrounded by an optically less dense area which will be referred to as the halo. The nucleoli of the young nuclei are spherical and occupy a more or less central place within the halo (Figs. 1–3), while those of the old nuclei are lens-shaped and occupy a polar position (Figs. 4, 5). The nucleolus rotates slowly within the halo, and the entire nucleus also shows slow movements within the cell. A true nuclear membrane has not been observed surrounding these nuclei.

Division of several of the living nuclei in the germinating basidiospores and hyphal tips has been observed, but the divisional sequence could not be photographed because of the movement of nuclei within the cell and the long exposure needed for phase photomicrography. The movements of the numerous vacuoles, granules, and mitochondria, contained within the cells, also hinder the observation of the living nuclei. At the beginning of the division, the optical density of the halo fluctuates considerably. At the same time the nucleolus, which is in a state of agitation, becomes increasingly smaller and finally disappears. The halo then increases in size, becomes elongated, and later constricts at the mid-region to form two small, oval-shaped halos which remain attached. A new nucleolus appears in each of the two halos as a tiny, dot-like, round, greyish body which enlarges rapidly. The two sister nuclei, each consisting of a nucleolus surrounded by a halo, then move apart. It takes approximately 8 to 10 minutes from the time the nucleolus disappears until it appears again in the daughter nucleus as a tiny dot.

### II. Stained Preparations

The cells of the vegetative mycelium of *C. praticolum* are multinucleate (Fig. 6) and those of the hymenium regularly binucleate. The vegetative cells of some strains of the closely related *Rhizoctonia solani* (*C. filamentosum* (Pat.) Olive) are also reported to be multinucleate (10, 17). The number of nuclei per vegetative cell varies from 4 to 25. The ungerminated basidiospores contain

one to four nuclei, the binucleate condition predominating (15).

Nuclei vary greatly in shape and size. Interdivisional nuclei in the cells of growing hyphae and basidiospores are smaller and usually spherical to subspherical (Fig. 7). In the cells of older hyphae they are larger, spherical to elongate (Fig. 6), or sometimes irregular in shape. The young nuclei, which are the products of recent division, are usually elongated and drawn out into a tail at one end (Figs. 13, 28). Several nuclei were observed wedged in the septal pores while migrating from one cell to another. Such nuclei appear dumbbell-shaped (Fig. 32). Sanford and Skoropad (17) report migration of nuclei through septal pores to be of common occurrence in *R. solani*, and consider this as one of the possible causes of variability in the number of nuclei in the cells of the vegetative hyphae.

*Nuclei in the Mycelium in Haematoxylin Preparations*

Nuclei stained with haematoxylin look similar to the living nuclei observed with the phase-contrast microscope. The interdivisional nucleus (Figs. 6, 7) consists of a black, round nucleolus, wholly or partly surrounded by a grey-colored area which corresponds to the halo of the living nucleus and to the site of Feulgen-positive chromatin in the Feulgen preparations (Figs. 36, 37). The nucleolus, which in living nuclei occupies a central position within the halo, was observed in all the stained preparations to occupy a polar position in relation to the chromatin.

Prior to the division of the nucleus, the nucleolus is no longer discernible and the chromatin acquires greater affinity for haematoxylin. The chromatin at this stage is not homogeneous, and has light-stained areas interspersed with dark-stained areas. The chromatin later increases in size, becomes elongated, and then constricts in the mid-region into two small portions which remain attached (Fig. 8). A new nucleolus then appears as a black, tiny dot within any part of each of the chromatin halves (Fig. 9), and increases rapidly in size (Figs. 10, 11). Following the development of the nucleoli, the two chromatin portions, which are still attached to each other by a narrow band of chromatin, pull apart (Figs. 11, 12). The sister nuclei at the end of the division are elongated, each consisting of a dark nucleolus surrounded by light chromatin (Fig. 13). Later they assume their regular spherical to subspherical outline.

*Nuclei in the Mycelium in HCl–Giemsa and Feulgen Preparations*

Nuclei stained by Feulgen and by HCl–Giemsa are very similar, but they differ in appearance from those stained in haematoxylin. This is in agreement with the findings of others (2, 3, 4, 5, 6, 7, 8, 13, 14). The crescent or cup-shaped chromatin, which stains lightly in haematoxylin, is deeply stained in the HCl–Giemsa and Feulgen preparations (Figs. 15, 16, 36, 37). The nucleolus, which stains deeply in haematoxylin, is Feulgen-negative, and can be seen in some of the overstained HCl–Giemsa preparations as a faintly stained body cupped within the concavity of the chromatin (Figs. 17, 18, 19).

During nuclear division in the HCl–Giemsa preparations, the nucleolus becomes progressively smaller and finally disappears (Figs. 19, 20). Following this, the chromatin becomes loosely granular, elongates to become bar-shaped, and then constricts at the mid-region (Figs. 21, 23, 24, 25, 39). At the end

of the division the two extremities pull apart, separating into two densely stained portions of chromatin (Figs. 26, 27, 28, 39). As observed in the haematoxylin preparations, the newly formed nucleoli could also be seen in some Giemsa preparations as faintly stained bodies in the separating chromatin extremities (Figs. 29, 30). A few figures were observed both in Feulgen and lightly stained Giemsa preparations, suggesting that the chromatin becomes concentrated on the periphery of the nucleus in the form of a ring at the beginning of the division, and divides, presumably into two equal portions, before the constriction of the nucleus (Figs. 22, 38). This could be the device by means of which the sister nuclei receive half of the chromatin material at the end of the nuclear division.

*Nuclei in the Basidiospores*

It has been shown formerly (15) that most of the basidiospores of *C. praticolum* are initially uninucleate. This single nucleus divides early, while the basidiospores are still attached to the sterigmata, to give rise to the binucleate condition found in the mature basidiospores. A number of HCl–Giemsa and haematoxylin preparations were studied to discover the mode of division of this original nucleus in the young basidiospores. The basidiospores are easily detached from the sterigmata, and are washed away during staining operations. This rendered the detailed study of the full divisional sequence in the young basidiospores difficult. Several divisional figures were obtained, which strongly suggest that, like the nuclei in the vegetative mycelium and germ tubes, the division of the original nucleus in the basidiospores also occurs by the elongation, constriction, and pulling apart of the chromatin extremities (Figs. 33, 34, 35).

Chromosomes or chromosome-like structures have not been observed in the nuclei of basidiospores, germ tubes, or mycelium of *C. praticolum* during division or at any other stage. It is interesting to note that nuclei with chromosomal filaments in reticulate arrangement were observed in the hymenial cells, and also in the cells of the short side branches which bear the hymenial cells, both in haematoxylin and in HCl–Giemsa preparations (Figs. 14, 31). Similar arrangement of chromosomal filaments has been previously reported in the two haploid nuclei before their fusion in the basidium (15). The area occupied by the chromosal filaments of these nuclei corresponded with the site of the chromatin of the vegetative nuclei.

## Discussion

Like the vegetative nuclei of *Mucor hiemalis* and *M. fragilis* (13), *Phycomyces blakesleeanus* (14), *Blastomyces dermatitidis* (2), *Endogone sphagnophila* (3), *Neurospora crassa* (4), *Gelasinospora tetrasperma* (5), *Schizophyllum commune* (6), *Phyllosticta* sp. (8), and some species of Saprolegniaceae (7), the nuclei of *C. praticolum* in the vegetative hyphae and in the basidiospores do not divide in a manner comparable to the classic mitotic division of the higher plants. Instead, the chromatin material of the nucleus divides by elongation, constriction, and separation of the two parts without the aid of a metaphase plate or spindle fibers. Chromosomal filaments have not been observed in these nuclei. As suggested by Robinow (13), and later Bakerspigel (3, 6, 7), it is

possible that the chromatin granules of the vegetative nuclei represent the chromosomes. The observed arrangement of the chromatinic granules in two separate portions on the periphery of the nucleus at the beginning of its division (Figs. 22, 38) possibly occurs after duplication and segregation of the granules. At the end of the nuclear division the sister nuclei possibly receive equal portions of the chromatin.

As in the vegetative mycelium of *B. dermatitidis* (2), *S. commune* (6), *Phyllosticta* sp., *Ophiostoma fimbriata*, and *Scopulariopsis brevicaulis* (8), the nucleolus disappears early during the division of the vegetative nucleus in *C. praticolum*, and the new nucleoli arise in the maturing sister nuclei. In these first five fungi, the nucleoli become separated from the chromatin early in division, and can be seen lying free in the cytoplasm till they degenerate completely. In the absence of any evidence to that effect in *C. praticolum*, it is more likely that the nucleolar material, instead of being bodily discarded into the cytoplasm, becomes diffused either in the remaining part of the nucleus, or in the surrrounding cytoplasm. The fact that the nucleoli were observed to become progressively smaller until they finally disappear from the nuclear chromatin is suggestive of this.

According to Robinow (13, 14) and Bakerspigel (2, 3, 4, 5, 6, 7, 8), the chromatin of the vegetative fungal nuclei has no marked affinity for haematoxylin, and they consider this stain to be useful for the study of the behavior of the nucleolus but not for chromatin. In the present study, haematoxylin was found equally useful for the study of the behavior of the nucleolus and the chromatin of the vegetative nuclei of *C. praticolum*. The HCl–Giemsa and Feulgen techniques, though superior to haematoxylin for the study of the details of the structure and behavior of chromatin, do not stain the nucleolus satisfactorily.

To date the cytology of the basidiospores, the mycelium, and the basidium has been studied in *C. praticolum*. Chromosomes have not been discerned in the nuclei of basidiospores and of mycelium, and these nuclei divide in a manner different from normal mitosis. But chromosomes have been observed in the haploid nuclei in the basidium, and the diploid nucleus in the basidium undergoes normal meiosis (15). Chromosomal filaments in reticulate arrangement have also been observed in the nuclei of the hymenial cells, and in the cells of the short side branches which bear the hymenial clusters (Figs. 14, 31). These nuclei with chromosomal filaments are similar to the haploid nuclei observed in the basidium (15). The cytology of the hymenial cells and the short side branches remains to be studied to complete the investigations on the behavior of nuclei throughout the life cycle of *C. praticolum*. It is likely that this study may reveal where the transition occurs between vegetative nuclear division of the type described here, and normal mitosis.

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NOTE: Figs. 1–39 and explanation of figures follow.

## Explanation of Figures

Figs. 1–5. Living nuclei; phase-contrast microscopy (×2000). Figs. 6–14. Iron alum haematoxylin (×2000).

Figs. 1–3. Living nuclei in basidiospores and germ tubes.

Figs. 4, 5. Living nuclei in old hyphal cells.

Fig. 6. Nuclei of a multinucleate, old hyphal cell.

Fig. 7. Nucleus in the germ tube showing a dark, small nucleolus and light-colored chromatin.

Fig. 8. Beginning of division. Constricted chromatin with no nucleoli.

Fig. 9. Development of new nucleoli in the two portions of constricted chromatin.

Fig. 10. Increase in size of the new nucleoli in the constricted chromatin. Arrows point to the nucleoli.

Fig. 11. Sister nuclei beginning to pull apart. Arrows point to the nucleoli.

Fig. 12. Separation of sister nuclei continuing.

Fig. 13. End of division. Young, elongated nuclei each with a nucleolus and a portion of chromatin.

Fig. 14. Nucleus in the hymenial cell showing a dark nucleolus and chromosomal filaments in reticulate arrangement.

Figs. 15–29. HCl–Giemsa (×2000).

Figs. 15, 16. Nuclei in hyphal cells. The concavity in the stained chromatin indicates the position of the unstained nucleoli.

Figs. 17, 18. Nuclei showing faintly stained nucleoli cupped within the concavity of the deeply stained chromatin.

Figs. 19, 20. Nuclei at the beginning of division. Note the elongated, granular chromatin and progressive decrease in the size of nucleoli.

Fig. 21. Elongated, granular chromatin of nuclei ready to divide. Note the absence of nucleoli.

Fig. 22. Nuclei showing concentration of chromatin on the periphery at the beginning of division.

Figs. 23, 24. Constriction of chromatin in the mid-region.

Fig. 25. Pulling apart of the chromatin extremities of a nucleus nearing end of division.

Figs. 26, 27. The extremities of the constricted chromatin shown separating near the end of division.

Fig. 28. End of division. The densely stained extremities completely separated.

Fig. 29. Separating chromatin extremities with newly formed, lightly stained nucleoli.

Figs. 30–35. HCl–Giemsa (×2000).

Fig. 30. Separating chromatin extremities with newly developed nucleoli.

Fig. 31. Nucleus in the cell of short side branches which bear the hymenial clusters showing a reticulum of chromosomal filaments. Compare with Fig. 14.

Fig. 32. Nucleus fixed while migrating through septal pore.

Fig. 33. Elongated chromatin of the single nucleus prior to its division in the basidiospore on left.

Fig. 34. Constricted chromatin of the single nucleus in a germinating basidiospore.

Fig. 35. Separation of the chromatin extremities at the end of division of the single nucleus in the basidiospore on right.

Figs. 36–39. Feulgen (×2000).

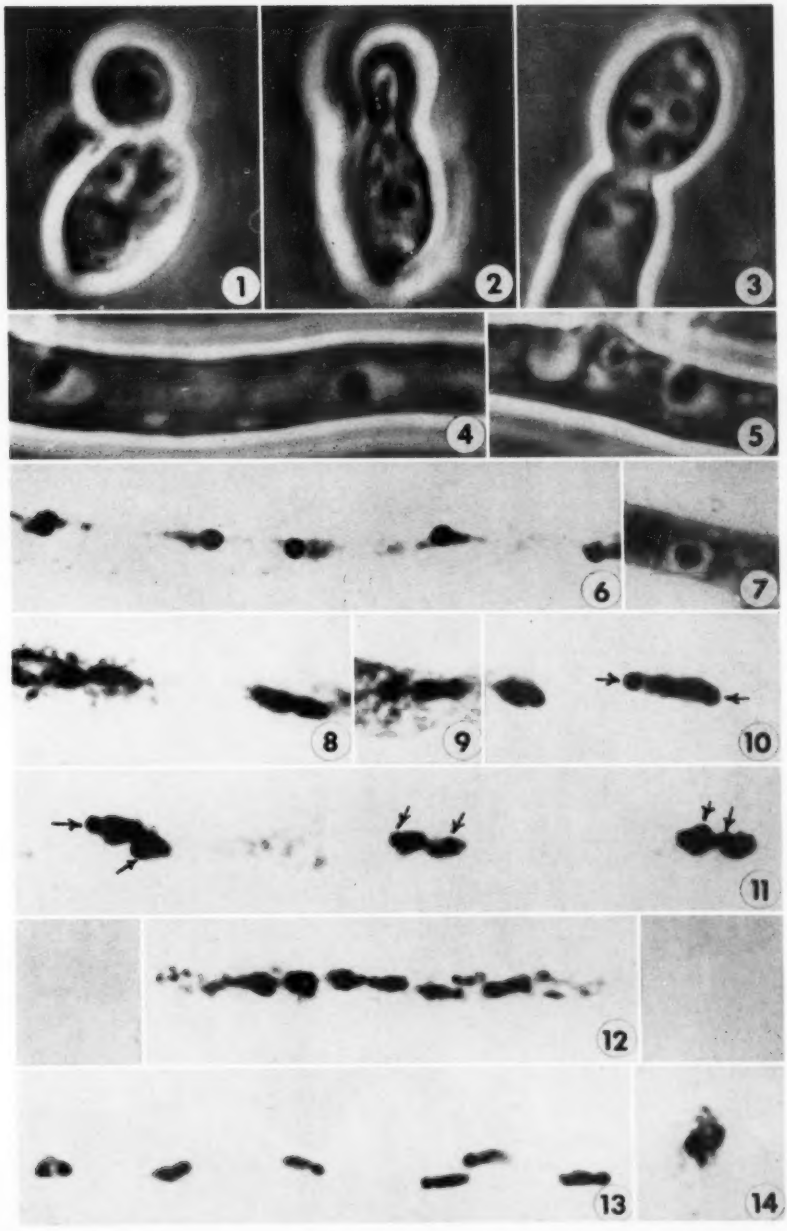
Fig. 36. Mature nuclei in the cells of older hypha.

Fig. 37. Young nuclei. Note the concentration of chromatin on the periphery of nucleus on left.

Fig. 38. Nucleus showing arrangement of chromatic granules on the periphery at the beginning of division.

Fig. 39. Constricted chromatin of a dividing nucleus in the lower part of hyphal tip is shown pulling apart. In the upper part of the hyphal tip can be seen the two chromatin extremities lying separated at the end of division.

PLATE I

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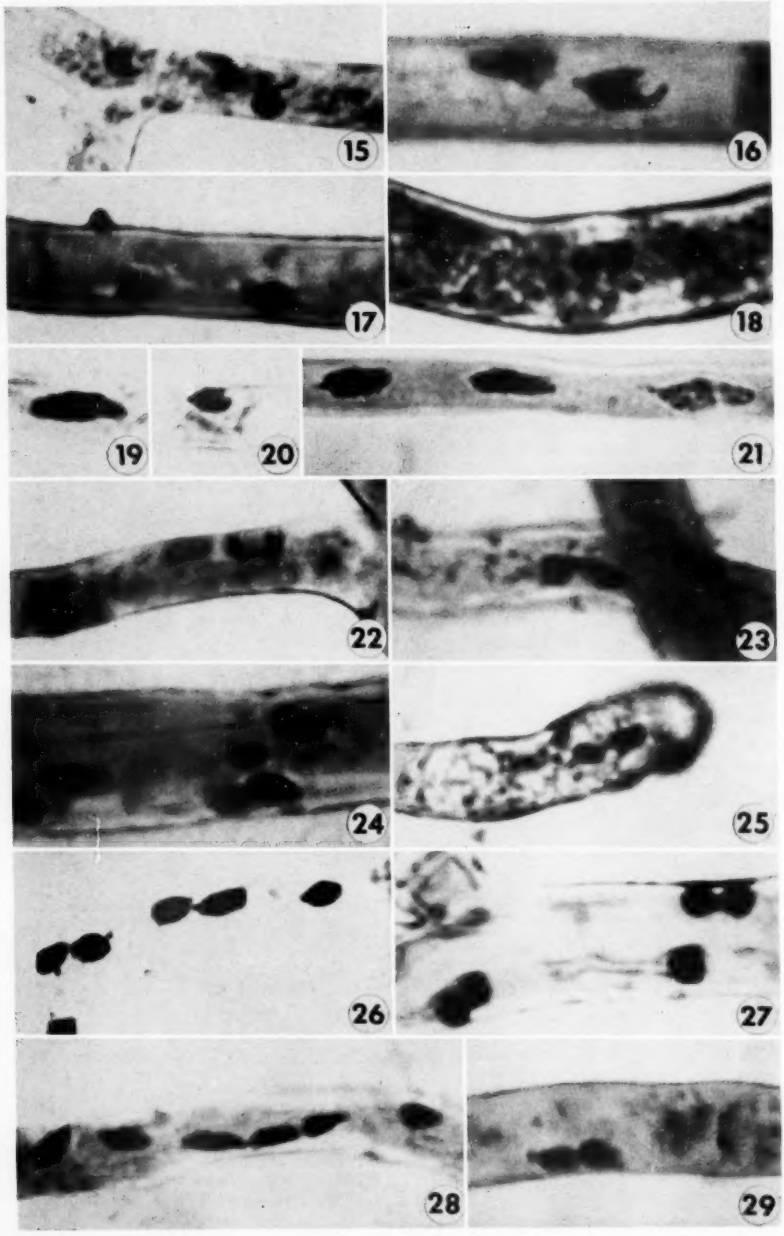


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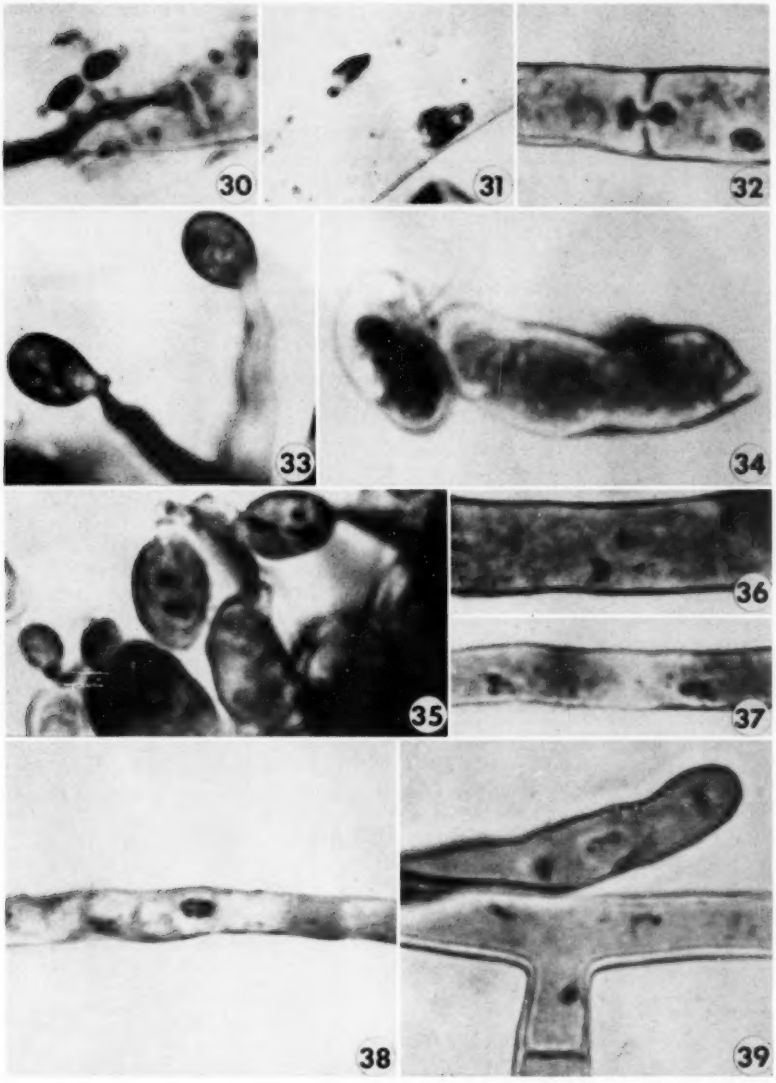


Saksena—Can. J. Botany

# CYTOLOGICAL STUDIES IN BROMUS SPECIES, SECTION BROMOPSIS[1]

M. R. HANNA[2]


## Abstract

Chromosome numbers and meiotic behavior were determined in clones of *Bromus inermis*, *Bromus pumpellianus*, and the *Bromus erectus* complex. Clones of *B. inermis* were octoploid ($2n = 56$) or near-octoploid, one aneuploid having $2n = 54$ chromosomes. *B. pumpellianus* clones were octoploid, with one bearing a B chromosome. *B. erectus*-type clones had 56, 59, 65, or 70 chromosomes. Meioses varied widely among plants in extent of irregularity. Meiotic behavior was studied in detail and the sequential interrelationships of irregularities at meiotic stages analyzed. Significant or highly significant positive correlations were obtained for the *B. pumpellianus* clones between percentages of non-stainable pollen and frequencies of metaphase I univalents, anaphase II laggards, and quartet micronuclei. These relationships were non-significant for the *B. inermis* and *B. erectus*-type clones. A significant negative correlation was determined between percentage of non-stainable pollen and percentage of seed set following open pollination, but other relationships between meiotic irregularities or pollen stainability and seed-setting characteristics were non-significant. The wide range in chromosome number and meiotic behavior encountered in material commonly distributed on this continent as "*B. erectus*" may be due to interspecific hybridization in nurseries in which *Bromus* introductions are grown. Interspecific hybridization may be partially responsible also for aneuploidy and meiotic irregularity in *B. inermis*.


## Introduction

Smooth bromegrass, *Bromus inermis* Leyss., is grown extensively for hay and pasture, and its value is widely recognized. Programs designed to increase the agronomic usefulness of brome have generally been confined to breeding and selection within the species. However, several closely related polyploid *Bromus* species have desirable characteristics that might profitably be incorporated into *B. inermis*. Among these features are the higher seed weight of *Bromus pumpellianus* Scribn. and the seedling vigor of *Bromus erectus* Huds. The cytological studies reported here were carried out in conjunction with a series of interspecific hybridizations between clones of *B. inermis*, *B. pumpellianus*, and the *B. erectus* complex.

## Review of Literature

An octoploid chromosome number, $2n = 56$, has been reported most commonly in *B. inermis* (3, 7, 11, 17, 18, 26, 27). Tetraploid, hexaploid, and decaploid counts have also been recorded (10, 14, 15, 18, 28). Recent studies (23, 27) indicate that aneuploid numbers in the range of 54 to 58 somatic chromosomes are rather frequent. Chromosome fragments have been described by several workers (11, 19, 20, 23).

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There is great variability among clones of *B. inermis* in the degree of meiotic irregularity. Multivalent associations, univalents, lagging chromosomes, bridges, and micronuclei have been described frequently (3, 7, 8, 10, 14, 18, 20, 23, 24, 25). Attempts to correlate meiotic behavior and pollen fertility with seed setting have met with varying success. Adams (1) failed to obtain significant relationships between meiotic irregularities and seed setting following either self- or open pollination. Schertz and Murphy (23) found open-pollinated fertility to be negatively correlated with percentages of anaphase I sporocytes containing lagging chromosomes, and percentages of spore quartets containing micronuclei. Data compiled by Nielsen (18) on 39 *B. inermis* clones demonstrated striking inverse relationships between the degree of meiotic irregularity at all stages of meiosis and percentage of seed setting under self- and open pollination. Percentage of pollen abortion was also closely correlated with frequency of meiotic aberrancies.

*Bromus pumpellianus* and *B. erectus* have not been well characterized cytologically. Stähli (28) reported 42 as the somatic chromosome number of *B. pumpellianus* but more recent studies have usually revealed 56 (3, 7, 9). A series of chromosome numbers, predominantly euploid and ranging from 42 to 84, have been recorded in *B. erectus* (3, 7, 12, 13, 28). Data on meiotic behavior in both species are somewhat fragmentary, but the same types of irregularities are encountered as in *B. inermis* (3, 7, 12, 13).

Cytotaxonomic relationships among the three species have not been defined clearly. They intercross rather readily and show considerable chromosome homology (7).

## Materials and Methods

Five clones of *B. inermis* (I-1, I-2, I-3, I-4, I-8) were selected from nurseries at Madison and three clones (I-11, I-12, I-13) from among a group of Alaskan accessions. The latter three plants were tentatively identified as *B. inermis* by Mr. J. R. Swallen, Smithsonian Institution, Washington, D.C. However, they had been collected in Alaskan locales where *B. inermis* and *B. pumpellianus* are associated, and they may have been hybrids between the two species. Despite their uncertain taxonomic position they have been grouped for convenience with the *B. inermis* clones. Six Alaskan clones (C91-2, C96-2, C97-29, C98-5, C98-9, C98-17) were chosen as representative *B. pumpellianus* specimens. An $F_1$ hybrid (P-4) between *B. pumpellianus* clones from Fort Saskatchewan, Alberta, and Palmer, Alaska, was also selected, although Mr. Swallen regarded it as "slightly atypical" of *B. pumpellianus*. Five introductions (E-1, E-2, E-3, E-4, E-5) received as *B. erectus* were also examined by Mr. Swallen, who concluded that none could be considered truly representative of the species. Consequently, they were designated "*B. erectus*-type" clones since the morphological characteristics of *B. erectus* predominated in them.

Root tips were obtained from clones grown in vermiculite in the greenhouse. Collections were made in late afternoon or evening and the root tips placed in .002 *M* 8-hydroxyquinoline solution in a refrigerator overnight. Squash preparations in aceto-orcein were made the following day. They were made permanent by freezing, removing the cover slip, immersing the slide in absolute alcohol, and remounting in Euparal. For the studies of microsporogenesis,

panicles at various stages of development were obtained from the field or greenhouse, fixed in Carnoy's 6:3:1 solution for 24 to 48 hours, and stored in 70% alcohol. Smear preparations of anthers were made in acetocarmine and heated briefly over a steam bath. Slides were made semipermanent according to the Venetian turpentine schedule outlined by Wilson (30).

Where possible, meiotic data were based on a minimum of 100 sporocytes, sporad pairs, or quartets from each clone at each of eight selected stages. Fixations for pollen examinations were made in Farmer's 3:1 solution. Estimates of pollen fertility were based on the staining reaction of pollen grains in 2% IKI solution in lactophenol.

Spikelets from plants in the field were selected at random for determinations of self- and open pollinated fertility. Several panicles per plant were placed in parchment bags to enforce selfing.

## Results and Discussion

### *Chromosome Counts*

Oxyquinoline pretreatment of root tips caused pronounced shortening of chromosomes, and aided chromosome spreading. Exact counts were obtained from 13 clones and approximate counts from seven others (Table I). Root-tip counts were not considered precise until the same number had been determined with certainty in several intact cells. Meiotic counts were obtained

TABLE I

Chromosome numbers ($2n$) of *Bromus inermis*, *B. pumpellianus*, and *B. erectus*-type clones

| Species and clone | Chromosome number determined from: | |
|---|---|---|
| | Root-tip mitosis | Microsporogenesis |
| *B. inermis* | | |
| I- 1 | 56 | 56 |
| I- 2 | 56 | 56 |
| I- 3 | —* | (56)† |
| I- 4 | (54–55) | (56) |
| I- 8 | 54 | (54) |
| I-11 | 56 | (56) |
| I-12 | (54–55) | (54) |
| I-13 | 56 | 56 |
| *B. pumpellianus* | | |
| P-4 | 56 | 56 |
| C91- 2 | — | 56 |
| C96- 2 | — | (56) |
| C97-29 | — | 56 |
| C98- 5 | — | (56) + B |
| C98- 9 | — | (56) |
| C98-17 | — | 56 |
| *B. erectus* complex | | |
| E-1 | 65 | (65) |
| E-2 | — | (56) |
| E-3 | 70 | 70 |
| E-4 | 59 | (57–58) |
| E-5 | 70 | (70) |

*Chromosome number not determined.
†Figures in parentheses represent approximate counts.

primarily from well-spread anaphase I stages; accurate counts at other meiotic stages were rarely recorded. There was no evidence of a difference in chromosome number between reproductive and somatic tissues in any plant.

The 54 chromosomes of the aneuploid plant I-8 included five SAT-chromosomes (Fig. 1). Schulz-Schaeffer (26) found six SAT-chromosomes in octoploid *B. inermis*, a feature observed in the present study in clones I-1, I-2, and I-11. Evidently, one of the two missing chromosomes of I-8 was an SAT-chromosome. Cytologically, the five *B. erectus*-type plants constituted a rather heterogeneous group (Table I). Figures 2 and 3 show somatic mitoses in the 59- and 65-chromosome aneuploids of this group. All but one of the seven *B. pumpellianus* plants appeared to possess 56 somatic chromosomes. The B chromosome observed in clone C98-5 will be discussed subsequently.

*Meiotic Observations*

The difficulties in making thorough cytological analyses in *B. inermis* and other Bromopsis polyploids have been emphasized previously (3, 6, 8, 10, 18). Chromosome clumping and stickiness, and the large numbers of chromosomes, make detailed studies of pairing relationships unreliable. These findings were confirmed in all plants examined during this study. Distinctions between true multivalent associations and adnation of chromosomes as a result of "stickiness" were particularly hard to make. Consequently, analysis of such associations was considered impracticable. All stages were examined in as much detail as possible but emphasis was placed on observations at metaphase I and subsequent stages. Data compiled for the various clones are presented in Table II.

*(a) Bromus inermis*

The course of meiosis was essentially as described by Elliott (7), Nielsen (18), and others. The meiotic behavior of the three Alaskan clones (I-11, I-12, I-13), which have been included in the *B. inermis* group, was not markedly different from the remaining clones.

Clear pachytene stages were rarely observed, but inversion loops, translocation configurations, and unpaired chromosome segments were noted in some cells from almost all clones. Univalents, trivalents, quadrivalents, and a few larger associations appeared at diplotene and diakinesis, although bivalents predominated in all plants. Differences in pairing relationships among clones of this species could not be distinguished.

There was a wide range in the frequency of metaphase I univalents, which constituted the most common irregularity at this stage. Most univalents remained unoriented, while the oriented chromosomes were generally associated as closed or open bivalents (Fig. 4). Chain and ring quadrivalents (Fig. 4), trivalents, and other groupings were again apparent in low frequencies. An anomalous characteristic of 20% of metaphase I cells of clone I-2 was the presence of one to several "stretched" or elongated open bivalents, similar to those described by Walters (29) in certain interspecific *Bromus* hybrids. An aberrant sporocyte from clone I-12 contained what appeared to be the equivalent of approximately 14 chromosomes (Fig. 5).

The most frequent irregularity at anaphase I was the lagging and subsequent division of univalent chromosomes into half-univalents in the interpolar

TABLE II

Average frequencies of irregularities at successive stages of microsporogenesis, and percentages of non-stainable pollen in *B. inermis*, *B. pumpellianus*, and *B. erectus*-type clones

| Species and clone number | Metaphase I, univalents | Anaphase I | | Telophase I, laggards | Dyad, micronuclei | Metaphase II, micronuclei | Anaphase II | | Telophase II, laggards | Quartet, micronuclei | Pollen, % non-stainable |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Half-univalent laggards | Bridges and bridge-fragments | | | | Laggards | Bridges | | | |
| *B. inermis* | | | | | | | | | | | |
| I-1 | .71 | .85 | .06 | .24 | .20 | .21 | .58 | .22 | .41 | .66 | 3.5 |
| I-2 | .67 | 1.04 | .02 | .36 | .31 | .33 | .79 | .06 | .61 | .75 | 1.8 |
| I-3 | 1.03 | 1.98 | .04 | .48 | .55 | .59 | 2.33 | .03 | 2.18 | 2.28 | 9.8 |
| I-4 | 1.10 | 1.70 | .12 | .54 | .49 | .57 | 1.59 | .13 | 1.72 | 1.74 | 13.1 |
| I-8 | 2.52 | 4.61 | .09 | .90 | .99 | 1.47 | 4.09 | .09 | 3.65 | 4.59 | 3.8 |
| I-11 | .20 | .49 | .17 | .51 | .22 | .22 | .42 | .25 | .81 | .37 | 2.0 |
| I-12 | 1.39 | 2.43 | .03 | 1.55 | .96 | .90 | .63 | None | 1.04 | 1.34 | 2.9 |
| I-13 | .50 | 1.07 | .04 | .73 | .96 | .56 | .50 | None | .56 | .53 | —§ |
| *B. pumpellianus* | | | | | | | | | | | |
| P-4 | .62 | .69 | .12 | .22 | .29 | .43 | 1.04 | None | .69 | .59 | 34.0 |
| C91-2 | .10 | .32 | .05 | .27 | .40 | .32 | .17 | None | .10 | .08 | 1.7 |
| C96-2 | .12 | .12 | .02 | None | .06 | .05 | .26 | .05 | .21 | .15 | 4.8 |
| C97-29 | .13 | .37 | .02 | .13 | .14 | .34 | .23 | .02 | .11 | .09 | 5.3 |
| C98-5* | .59 | .56 | .28 | .29 | .47 | .37 | .69 | None | .60 | .50 | 14.5 |
| C98-9 | .41 | 1.54 | .08 | .24 | .12 | .16 | .70 | .10 | .53 | .54 | 10.3 |
| C98-17 | .05 | .07 | .07 | .11 | .12 | .20 | .24 | None | .10 | .07 | 1.7 |
| *B. erectus* complex | | | | | | | | | | | |
| E-1 | 2.46† | 1.47‡ | .14 | 1.71 | 1.30 | .87 | .36 | .14 | .64 | .74 | —§ |
| E-2 | 1.76 | 3.05 | .11 | 2.04 | 2.13 | 1.75 | 1.63 | .11 | 1.15 | 1.16 | 15.2 |
| E-3 | .06 | .12 | .02 | .19 | .15 | .11 | .16 | .03 | .17 | .11 | 9.1 |
| E-4 | 1.91 | 3.21 | .03 | .39 | .21 | .75 | 2.71 | .21 | 1.60 | 1.26 | 1.8 |
| E-5 | .55 | .38 | .06 | .49 | .44 | .06 | .25 | .04 | .42 | .53 | 56.7 |

*Excluding data on B chromosome.
†Including .44 ring univalents per cell.
‡Since univalents do not divide in clone E-1, this figure represents the frequency of univalent laggards instead of half-univalent laggards.
§Data not obtained.

region. Few univalents failed to divide at this stage, and large numbers of half-univalents occurred in some cells (Fig. 6). All excluded bodies at late anaphase I and telophase I were originally considered to be derived solely from laggard half-univalents or univalents. However, differences detected among these exclusions led to a re-evaluation of their origin. Some seemed typically chromosomal in shape and staining properties, while others lacked a characteristic chromosomal appearance, were generally globular, and stained more darkly with acetocarmine. In size, the various exclusions were usually indistinguishable. The globules were most evident in sporocytes of clone I-8 but were seen occasionally in other plants. An attempt to trace back to earlier developmental stages of these globular formations revealed a sequence of events closely paralleling that described by Nielsen (20) in certain *B. inermis* plants. Some pachytene cells of clone I-8 and other plants had small accumulations of darkly stained material at several points along the prophase chromosomes. Although later prophase stages did not reveal clearly the progression of this globule formation, metaphase I sporocytes could be found in which globules appeared to be forming distally on one to several chromosome arms. None of the globules or droplets attained the size of some which Nielsen (20) reported, nor were his smaller "pepper structures" observed.

Anaphase I bridge and bridge-fragment configurations, the latter indicative of inversion heterozygosity, occurred with low frequencies. Some of the bridges without accompanying fragments may have been due to chromosome stickiness. Frequencies of dyad micronuclei in all clones were lower than frequencies of anaphase I laggards, indicating that large numbers of laggards were reincorporated into the dyad nuclei.

Chromosome stickiness at metaphase II was encountered in several clones. Sometimes the entire chromosome complement in paired sporads had a gummy, reticulate appearance (Fig. 7). Lagging chromosomes were common at anaphase II (Fig. 8), and second division bridges were noted in all but two clones. From 22 to 97% of the spore quartets in the eight clones contained one or more micronuclei or globules of various types. As was true of those at earlier stages, most of the excluded bodies were assumed to be of chromosomal derivation. However, they were not always uniform in appearance and

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FIGS. 1 to 3. Root-tip mitoses in *B. inermis* and *B. erectus*-type clones.
FIG. 1. Clone I-8: $2n = 54$; satellites indicated by arrows.
FIG. 2. Clone E-4: $2n = 59$.
FIG. 3. Clone E-1: $2n = 65$.
FIGS. 4 to 9. Microsporogenesis in *B. inermis*.
FIG. 4. Clone I-1: univalent and ring quadrivalent at metaphase I.
FIG. 5. Clone I-12: aberrant metaphase I sporocyte with a reduced chromosome number; $2n$ = approximately 14.
FIG. 6. Clone I-8: lagging half-univalents at anaphase I.
FIG. 7. Clone I-4: chromosome stickiness at metaphase II.
FIG. 8. Clone I-8: lagging chromosomes at early telophase II.
FIG. 9. Clone I-8: quartet with five condensed micronuclei and large granular micronucleus.
FIGS. 10 to 12. Microsporogenesis in *B. pumpellianus*.
FIG. 10. Clone C96-2: pachytene.
FIG. 11. Clone C91-2: diakinesis; "Kreuzbivalent" indicated by arrow.
FIG. 12. Clone C91-2: 18 closed and 10 open bivalents at metaphase I.

PLATE I

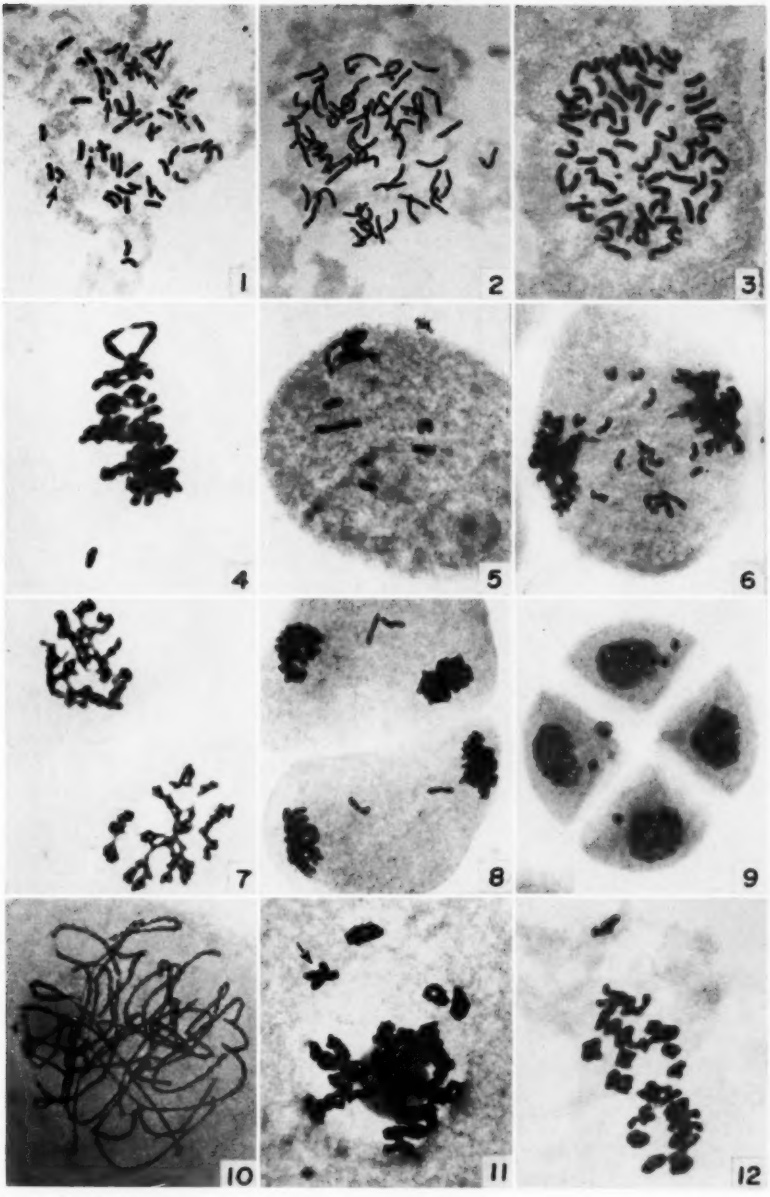


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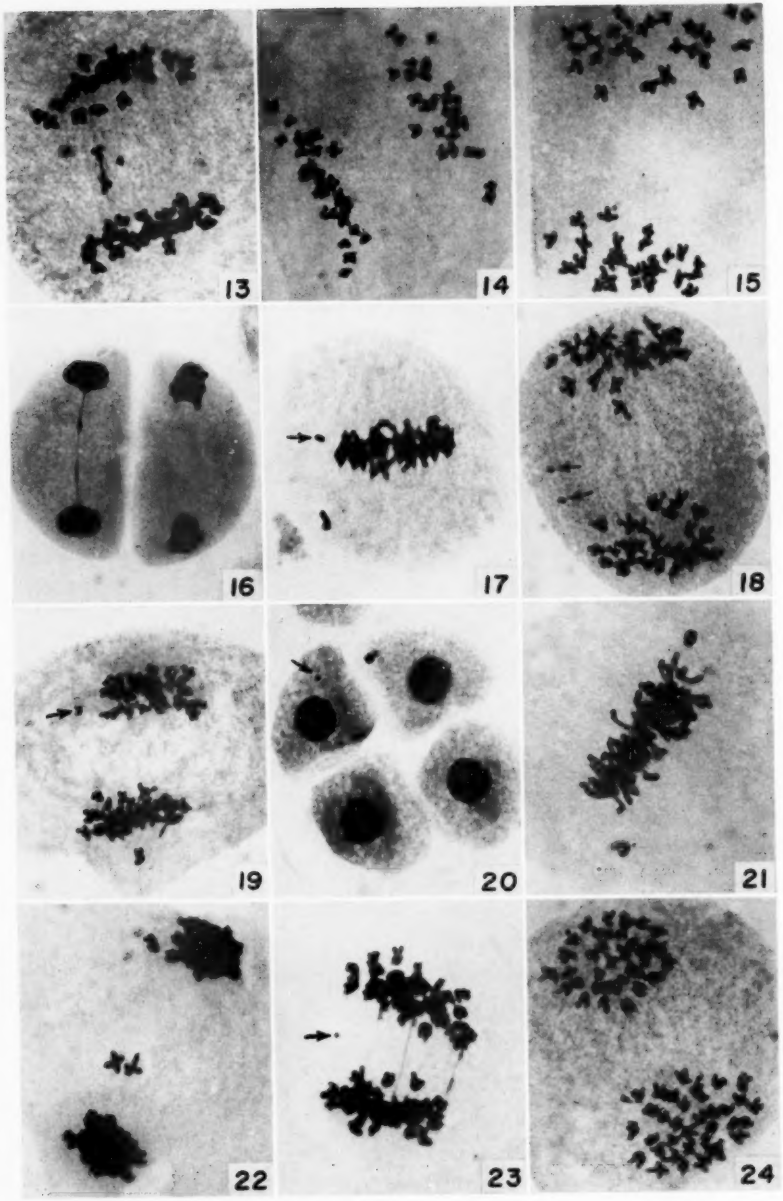
PLATE II

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staining properties (Fig. 9) and some may not have been chromosomal in origin. Pollen stainability was generally high; the high percentage in clone I-3 is notable in view of its aneuploid ($2n = 54$) chromosome complement and its extreme irregularity throughout meiosis.

*(b) Bromus pumpellianus*

The types of meiotic irregularities encountered in *B. pumpellianus* were similar to those described for *B. inermis*, but there was a marked difference between the two species in the over-all level of irregularity. Four of the seven *B. pumpellianus* clones were more regular throughout meiosis than any of the *B. inermis* clones. The remaining plants had levels of irregularity essentially the same as those of the more regular *B. inermis* plants.

Prophase stages in *B. pumpellianus* (Figs. 10, 11) were similar to those of *B. inermis* and were equally difficult to analyze. Some chromosome stickiness was observed but it was not pronounced. The degree of multivalent association appeared to be similar in the two species, a conclusion already drawn by Elliott (7). Occasional bivalents in diakinetic cells had an X-configuration (Fig. 11) much like those described by Schulz-Schaeffer (25) as "Kreuzbivalente". The cause of such configurations is undetermined.

Few metaphase I cells were well spread, but those that could be analyzed had 28 bivalents (Fig. 12). No metaphase cell of any clone had more than two univalents, and the average univalent frequency was low. Trivalents and quadrivalents were uncommon. All lagging chromosomes divided equationally at anaphase I. Bridges or bridge-fragment associations occurred in low frequencies (Fig. 13). A number of accurate chromosome counts were obtainable from anaphase I stages (Figs. 14, 15), possibly a reflection of the low incidence of stickiness. In Fig. 15 the chromosomes of clone C98-17 are shown undergoing a 27–29 anaphase I separation, which may have been the result of the unequal separation of a multivalent association. This was the only cell in which unequal disjunction could be demonstrated conclusively, although less suitable preparations yielded suspect examples.

Anaphase I laggards were slower to reach the poles in *B. pumpellianus* than in *B. inermis* and so were not as readily incorporated into daughter nuclei. An exception was found in clone C98-9, which had a frequency of only 0.12 micronuclei per dyad in spite of a frequency of 1.54 laggards per cell at anaphase I.

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FIGS. 13 to 20. Microsporogenesis in *B. pumpellianus*.
FIG. 13. Clone C98-9: dicentric bridge and acentric fragment at anaphase I.
FIG. 14. Clone C91-2: 28–28 chromosome separation at anaphase I.
FIG. 15. Clone C98-17: 29–27 chromosome separation at anaphase I.
FIG. 16. Clone C98-9: bridge at telophase II.
FIG. 17. Clone C98-5: B chromosome (arrow) and standard univalent at metaphase I.
FIG. 18. Clone C98-5: divided B chromosome (arrows) at anaphase I.
FIG. 19. Clone C98-5: undivided B chromosome (arrow) at late anaphase I.
FIG. 20. Clone C98-5: micronucleus formed from B chromosome (arrow) and standard micronucleus at quartet stage.
FIGS. 21 to 24. Microsporogenesis in *B. erectus*-type clones.
FIG. 21. Clone E-1: two univalents, including ring univalent, at metaphase I.
FIG. 22. Clone E-1: undivided univalent laggards at first division.
FIG. 23. Clone E-1: two bridges and single fragment (arrow) at anaphase I.
FIG. 24. Clone E-3: 35–35 chromosome separation at anaphase I.

The comparatively low level of irregularity characteristic of the early stages of meiosis in *B. pumpellianus* plants continued through the second division up to and including the quartets. Stickiness was observed in only a few metaphase II cells. Bridges were noted in sporads of three clones (Fig. 16).

Apart from the commonly encountered irregularities such as laggards, micronuclei, and bridges, there were fewer "miscellaneous" irregularities in *B. pumpellianus* such as unoriented bivalents, elongated bivalents, and other aberrancies seen occasionally in *B. inermis*. A B chromosome, described below, was noted in clone C98-5.

The meiotic behavior of the B chromosome was followed in detail. During prophase it appeared as an unpaired fragment about one-fifth the length of the majority of bivalents, and during metaphase I as a small univalent (Fig. 17). It was seen commonly as a dividing or divided laggard at anaphase I (Fig. 18), but in some cells it had passed undivided to one pole or appeared to be in the process of division after passage to the pole (Fig. 19). Small micronuclei, formed from either the divided or undivided B chromosome, could be located in some dyads. The excluded B chromosome, or its derived half-univalents, were seen occasionally during second division, and appeared as B micronuclei with a frequency of 0.43 per quartet (Fig. 20).

As far as is known, this is the first time that a B chromosome has been recorded in *B. pumpellianus*. Its meiotic behavior indicated that it was not an acentric fragment. The exact chromosome number of clone C98-5 was not determined, so it is not known if the B chromosome was present in addition to the normal octoploid complement or as a substitute for one of the normal chromosomes. However, in common with B chromosomes that have been described in other species (21), it did not pair with chromosomes of the normal complement.

In most species one or two B chromosomes do not produce obvious cytological disturbances or effects on fertility or phenotype, but such effects often become evident as the number of such chromosomes increases (5). There was no indication of whether or not the B chromosome in clone C98-5 influences meiotic regularity. Data on the transmissibility of the B chromosome are not available, but the presence of B micronuclei in quartets indicates a certain degree of meiotic elimination on the male side. Its postmeiotic behavior was not investigated to determine whether it had a non-disjunctive mechanism at one of the pollen mitoses, such as occurs in a large number of other grasses (4, 16, 21, 22). Further cytological and breeding studies would be necessary to provide details of the origin, behavior, and possible genetic effects of the B chromosome.

*(c) Bromus erectus Complex*

The variation in chromosome number among the five *B. erectus*-type plants was accompanied by equally extreme differences in meiotic behavior and irregularity. In the two 70-chromosome plants (E-3 and E-5) meiotic division was rather regular, while in the 65-, 59-, and (approximately) 56-chromosome plants it was highly irregular.

Pachytene stages provided little information. The chromosomes were generally so difficult to spread that it was impossible to distinguish even

between cells from the 56- or 70-chromosome plants. Stickiness or multivalent formation or both were widespread at diakinesis, and associations or adnations involving up to 8 or 10 chromosomes were common. As many as six or seven univalents were observed in metaphase I cells of clones E-1, E-2, and E-4. A ring univalent appeared in almost half the metaphase I sporocytes of clone E-1 (Fig. 21). None of the plants had appreciable frequencies of trivalents or quadrivalents at this stage, but chains and rings involving at least five or six chromosomes were noted in clone E-1.

The frequencies of lagging univalents at anaphase I corresponded in a general way to metaphase I univalent frequencies in all five clones. In four of these almost all univalent laggards divided at anaphase I, but in clone E-1 the laggards behaved in a manner unlike that recorded for any other among the 20 *Bromus* plants studied. In more than 100 sporocytes from clone E-1 analyzed at anaphase I, no clear example of a divided univalent was noted. Although many univalent laggards appeared to be in the process of division (Fig. 22), there was no evidence of a completed division. Further evidence of incomplete division was provided by the large size of micronuclei at the dyad stage, which suggests their derivation from undivided univalents. It is quite possible that the non-disjunctive process is gene controlled.

An aberrant characteristic of about 5% of late anaphase I or early telophase I sporocytes of clone E-2 was the occurrence of one or more lightly stained bodies lying between the polar chromosome groups. These were very similar to the globular "chondriosomes" described by Armstrong (2) in certain alfalfa polyploids. They may have represented a slightly different expression of the globules noted in some of the *B. inermis* plants. Globule formation of this latter type was seen occasionally in clones E-1 and E-4. Bridges and bridge fragments were observed in all plants (Fig. 23), although anaphase I stages in the two decaploid clones were generally regular (Fig. 24). Second division bridges also occurred in the five plants. Quartet micronuclei were again somewhat variable in size, shape, and staining properties, as in several of the *B. inermis* plants. However, all exclusions were considered in estimating frequencies of quartet micronuclei.

*Interrelationships of Meiotic Irregularities*

A complete picture of the pattern of meiotic behavior in the 20 *Bromus* clones cannot be gained solely from descriptive treatment of the cytological findings, or even from close examination of the quantitative data compiled in Table II. Correlation coefficients (Table III) were calculated in order to obtain some clarification of the sequential interrelationships among the various irregularities found throughout meiosis. These correlations were obtained between average frequencies of irregularities at given stages for the 20 clones as a group, and separately for the eight *B. inermis* clones and for the seven *B. pumpellianus* clones. Correlations for the *B. erectus*-type plants were not calculated separately because of the small number (five) of clones.

Metaphase I univalents are generally assumed to be the precursors of subsequent irregularities, such as anaphase laggards, and dyad and quartet micronuclei. Two of the correlations between frequencies of metaphase I

TABLE III

Correlations among average frequencies of irregularities at stages of meiosis in *B. inermis*, *B. pumpellianus*, and *B. erectus*-type clones

| Correlation between: | Twenty clones of *B. inermis*, *B. pumpellianus*, and *B. erectus* complex, $r$ (18 d.f.) | Eight clones of *B. inermis*, $r$ (6 d.f.) | Seven clones of *B. pumpellianus*, $r$ (5 d.f.) |
|---|---|---|---|
| Metaphase I univalents and anaphase I laggards | .56** | .98** | .58 |
| Metaphase I univalents and dyad micronuclei | .67** | .62† | .50 |
| Metaphase I univalents and metaphase II micronuclei | .83** | .94** | .52 |
| Metaphase I univalents and anaphase II laggards | .70** | .86** | .95** |
| Metaphase I univalents and quartet micronuclei | .72** | .94** | .96** |
| Anaphase I laggards and dyad micronuclei | .57** | .69† | .01 |
| Anaphase I laggards and anaphase II laggards | .81** | .88** | .62 |
| Anaphase I laggards and quartet micronuclei | .86** | .95** | .76* |
| Dyad micronuclei and metaphase II micronuclei | .86** | .80*† | .73 |
| Dyad micronuclei and anaphase II laggards | .30 | .41 | .29 |
| Dyad micronuclei and quartet micronuclei | .38 | .52 | .30 |
| Metaphase II micronuclei and anaphase II laggards | −.08 | .79* | .44 |
| Metaphase II micronuclei and quartet micronuclei | .67** | .88** | .32 |
| Anaphase II laggards and quartet micronuclei | .91** | .98** | .96** |
| Anaphase I bridges and anaphase II bridges | .17 | .74* | −.27 |

*Significant at the 5% level.
**Significant at the 1% level.
†Significant at the 1% level if data from clone P-3 are excluded.

and anaphase I univalents were highly significant. However, the majority of clones had anaphase I univalent frequencies varying from 0.02 to 0.99 per cell lower than the corresponding frequencies of metaphase I univalents, indicating that the significance of the correlations was not based on a 1:1 relationship of metaphase I univalents:anaphase I univalents. A paired "*t*" test on the data from all 20 clones was used to determine the significance of this reduced frequency of anaphase I univalents. The "*t*" value obtained was 2.91, exceeding tabled "*t*" at the .01 level of probability. This indicates that a highly significant proportion of the metaphase I univalents remained unaccounted for at anaphase I. The most likely explanation is that many of the univalents divided early enough to pass to the poles in conjunction with the normally dividing bivalents, thus escaping detection as laggards.

The correlation between metaphase I univalents and anaphase I laggards in the *B. pumpellianus* clones was non-significant, but approached significance when clone C98-9 was omitted from consideration. Instead of a reduced anaphase I univalent frequency, as observed in most clones, this plant had almost twice as many anaphase univalents as metaphase univalents. Sampling error may have been responsible, or laggards may have arisen from some additional source other than metaphase univalents.

The large number of highly significant correlations is the most obvious feature of Table III. For the 20 clones as a group, all eight relationships involving metaphase I univalents and anaphase I laggards were highly significant. The same is true for the *B. inermis* clones, except that the two correlations having dyad micronuclei as the second variable were non-significant unless the data from clone I-13 were excluded. This is a reflection of the apparent tendency in clone I-13 for approximately 90% of the lagging half-univalents to remain excluded as micronuclei in the dyads. Conversely, only 10% of the laggards reached the polar chromosome groupings in time to be reincorporated into the daughter nuclei. This reincorporation or "recovery" averaged 69% in the remaining seven *B. inermis* clones. A similar relationship can be calculated from meiotic data on 34 *B. inermis* plants studied by Nielsen (18). In these plants recovery averaged 70.5%, and all but four of the individual percentages fell within the range of 65–90%. Thus, there appears to be a rather consistent tendency for approximately two-thirds to three-quarters of lagging anaphase half-univalents in this species to reach the poles in time to be included in the dyad nuclei.

For the *B. pumpellianus* clones, only three of the correlation coefficients involving metaphase I univalents and anaphase I laggards were significant at the .05 or .01 levels. However, all were positive and only one was below .50. The low correlation between anaphase I laggards and dyad micronuclei was due to the distinct differences in recovery from first division lagging among the *B. pumpellianus* plants, ranging from 0–92%. Similarly, among the *B. erectus*-type plants recovery varied from 0–94%. Since the laggards in sporocytes of clone E-1 did not divide, recovery was calculated on the proportion of dyad micronuclei to anaphase univalents rather than to half-univalents. Recovery calculated in this way was only 12%.

In most of the *B. pumpellianus* and *B. erectus*-type clones in which recovery appeared to be low the initial frequency of laggards at anaphase I was also

low. Consequently, in such plants the ratio of anaphase I laggards to dyad micronuclei may have been affected more severely by small sampling errors in the estimation of these irregularities. For example, recovery of 50% based upon frequencies of 2.0 half-univalents per anaphase I cell and 1.0 micronucleus per dyad would be more reliable than a similar recovery percentage based on frequencies of 0.2 and 0.1, respectively. Recovery in clones with rather regular meioses therefore may have been higher than the data suggest.

The ultimate fate of dyad micronuclei is difficult to ascertain. Since the frequency of dyad micronuclei in some clones (particularly I-13, C91-2, E-1, and E-2) was considerably higher than the frequency of quartet micronuclei, it appears that many dyad micronuclei were either reincorporated into quartet nuclei or absorbed into the cytoplasm before the quartet stage. In the remaining clones the frequencies of quartet micronuclei either approximated the frequencies of dyad micronuclei or (more commonly) were appreciably higher. The absence of a consistent relationship between dyad micronuclei and quartet micronuclei is evidenced by the variability among the correlations between their frequencies.

All three correlations between frequencies of anaphase II laggards and quartet micronuclei were highly significant. The close approximation to a 1:1 ratio between these frequencies in most clones suggests that the quartet micronuclei originated almost exclusively from anaphase II laggards and that there was no second division recovery. This also tends to confirm the earlier suggestion that dyad micronuclei disappeared before quartet formation and did not contribute to the formation of quartet micronuclei.

The origin of the anaphase II laggards is also difficult to determine. They may have had no relationship to first division irregularities, or they may have arisen from those first division laggards that eventually reached the poles in time to be incorporated into the dyad nuclei. A similarity between the estimated frequency of these included half-univalent laggards and the frequency of anaphase II laggards would then suggest that the latter originated from the former. This hypothesis seems tenable for only about half the clones, while in most of the remaining clones there were more anaphase II laggards than can be accounted for in this way.

The foregoing discussion indicates that the interrelationships of the more common meiotic irregularities cannot be explained entirely on a simple basis. However, a tentative summary of the pattern of behavior can be advanced which seems to fit the data most satisfactorily:

(1) most metaphase I univalents appear at anaphase I as dividing laggards;

(2) a large proportion of the resultant half-univalents reach the poles in time to be incorporated in the dyad nuclei, while the excluded half-univalents form micronuclei in the dyads;

(3) the dyad micronuclei do not contribute appreciably to subsequent observed irregularities but probably are either reincorporated into quartet nuclei or are absorbed into the cytoplasm before the quartet stage;

(4) anaphase II laggards are derived in part from first division laggards that were not excluded as dyad micronuclei;

(5) quartet micronuclei are derived entirely from anaphase II laggards.

The highly significant correlations between frequencies of irregularities at meiotic stages indicate that within each clone there was a fairly consistent level of irregularity throughout meiosis. This is particularly true of the *B. inermis* clones. Frequencies of metaphase I univalents, anaphase I laggards, anaphase II laggards, and quartet micronuclei appear almost equally reliable as indicators of the general level of irregularity within each clone.

*Pollen Fertility and Seed Setting*

Correlations between percentages of non-stainable pollen and average frequencies of irregularities at several meiotic stages were obtained for the group of 18 plants of *B. inermis*, *B. pumpellianus*, and the *B. erectus* complex for which data on pollen stainability were recorded. Correlations were also determined separately for the *B. pumpellianus* plants and the *B. inermis* plants (Table IV).

TABLE IV

Correlations between average frequencies of meiotic irregularities and percentages of non-stainable pollen in *B. inermis*, *B. pumpellianus*, and *B. erectus*-type clones (Arc sine transformation of percentage data)

| Correlation between percentage non-stainable pollen and mean frequency of: | Eighteen clones of *B. inermis*, *B. pumpellianus*, and *B. erectus* complex, *r* (16 d.f.) | Seven clones of *B. inermis*, *r* (5 d.f.) | Seven clones of *B. pumpellianus*, *r* (5 d.f.) |
|---|---|---|---|
| Metaphase I univalents | −.35 | .16 | .91** |
| Anaphase I laggards | −.15 | .14 | .45 |
| Dyad micronuclei | .14 | .10 | .19 |
| Anaphase II laggards | −.09 | .34 | .94** |
| Quartet micronuclei | −.06 | .29 | .87* |

*Significant at the 5% level.
**Significant at the 1% level.

None of the five correlations between cytological irregularities and pollen abortion for the 18 clones as a group was significant. Four of the five correlation coefficients were negative. This is due primarily to the effect of several clones, such as P-4 and E-5, which were rather regular at meiosis but had 56.7 and 34.0% non-stainable pollen, respectively. Conversely, several plants such as I-8 and E-4 were highly irregular at meiosis but had high percentages of stainable pollen. The five correlations for the *B. inermis* plants were positive but non-significant. Only in *B. pumpellianus* were meiotic irregularities at metaphase I, anaphase II, and the quartet stage significantly correlated with pollen stainability. Although chromosomal irregularities appeared to be largely responsible for pollen abortion in *B. pumpellianus*, this was not true of the *B. inermis* and *B. erectus*-type plants. Pollen abortion in these plants may have been under genic control.

Correlations between cytological irregularities, including pollen abortion, and seed setting following self- and open pollination are summarized in Table V for the group of plants from which seed-setting data were obtained. All correlations between average frequencies of meiotic irregularities and

TABLE V

Correlations between average frequencies of meiotic irregularities and percentage of non-stainable pollen, and percentage of seed set following self- and open pollination in *B. inermis*, *B. pumpellianus*, and *B. erectus*-type clones (Arc sine transformation of percentage data)

| Correlation between: | d.f. | *r* |
|---|---|---|
| Metaphase I univalents and selfed seed set | 12 | .46 |
| Anaphase I laggards and selfed seed set | 12 | .22 |
| Dyad micronuclei and selfed seed set | 12 | .15 |
| Anaphase II laggards and selfed seed set | 12 | .23 |
| Quartet micronuclei and selfed seed set | 12 | .25 |
| Non-stainable pollen and selfed seed set | 10 | .08 |
| Metaphase I univalents and open-pollinated seed set | 12 | −.01 |
| Anaphase I laggards and open-pollinated seed set | 12 | −.10 |
| Dyad micronuclei and open-pollinated seed set | 12 | −.32 |
| Anaphase II laggards and open-pollinated seed set | 12 | −.33 |
| Quartet micronuclei and open-pollinated seed set | 12 | .21 |
| Non-stainable pollen and open-pollinated seed set | 10 | −.58* |

*Significant at the 5% level.

percentage of seed set following either self-pollination or open pollination were non-significant. The significant negative correlation between percentage of non-stainable pollen and percentage of seed set under open pollination indicated a relationship between male and female fertility.

*Aneuploidy, Meiotic Irregularity, and Hybridity*

As already indicated, only one of the *B. inermis* plants studied could be clearly defined as an aneuploid (I-8, with $2n = 54$ chromosomes). Clone I-8 was also the most irregular of the *B. inermis* plants, while the other two (I-4 and I-12) suspected of being aneuploids were also rather irregular. Conversely, the four plants with the lowest levels of irregularity were definitely established as being euploids with 56 chromosomes. Sigurbjörnsson *et al.* (27) and Schertz and Murphy (23) have already demonstrated conclusively that aneuploids are of rather frequent occurrence in this species, and have stated that they exhibit greater meiotic irregularity than euploids. Seventy-eight of the seventy-nine aneuploids among the 222 plants studied by Sigurbjörnsson *et al.* (27) and all 13 aneuploids among the 25 plants examined by Schertz and Murphy (23) had 54, 55, 57, or 58 somatic chromosomes. It is possible to explain such variation on the basis of the production of functional 27- and 29-chromosome aneuploid gametes. Schertz and Murphy (23) and others have occasionally recorded 27–29 anaphase I chromosome distributions in *B. inermis* (see also Fig. 15, showing a similar disjunction in *B. pumpellianus*). However, there are insufficient data to indicate whether or not such distributions actually lead to the production of functional aneuploid gametes. Hill and Carnahan (10) obtained no aneuploids among the progeny of a tetraploid *B. inermis* clone interplanted with normal octoploids, which suggests that in this case, at least, the functional *B. inermis* pollen from the octoploids contained 28 chromosomes. More critical data are needed on chromosome numbers in progenies of euploid and aneuploid plants.

In the past, *B. inermis* nurseries at Madison, and undoubtedly elsewhere, have frequently been grown adjacent to other *Bromus* species. In view of the degree of cross-compatibility between *B. inermis* and related polyploids, hybridization could occur readily. Aneuploidy and accompanying meiotic irregularity among progenies of *B. inermis* clones grown under these circumstances might therefore be attributable directly to such hybridization. The natural range of morphological variability in *B. inermis* may hinder recognition of hybrid characteristics unless progenies are examined carefully. Observations by Nielsen (personal communication) and Knowles (personal communication), at Madison and Saskatoon, respectively, suggest that introgression of *B. erectus* characteristics into *B. inermis* has occurred at these locations when adequate isolation has not been provided.

The range of chromosome numbers reported here and elsewhere among *B. erectus*-type plants emphasizes the heterogeneity that exists within the material available on this continent under the designation "*B. erectus*". Unpublished data by Nath and Nielsen suggest that much of this material falls into what is best regarded as the *B. tyttholepsis* complex. Other accessions in the Madison grass nurseries, tentatively identified as members of the *B. erectus* complex, have been examined by Nath and Nielsen. Some of the somatic chromosome counts recorded were 54, 56, 63, 65, 68, and 80. Again, hybridization between species or chromosome races may be largely responsible. Introductions from Europe, Asia Minor, and other areas frequently possess a long history of accession from station to station in the United States and Canada. Some of the *B. erectus*-type plants used in this study were derived from material introduced into North America almost two decades ago. These introductions probably passed through several generations of seed increase in the interim. Since introductions are usually maintained in nurseries that may include a wide variety of *Bromus* species and strains, seed harvested from such plants is of questionable parentage, even though it is distributed as "*B. erectus*". Further hybridization in later generations will give rise to added cytological and morphological variation. It is understandable that difficulty is experienced in categorizing such material taxonomically or cytologically.

The widespread meiotic irregularity among the *B. inermis* and *B. erectus*-type clones, particularly globule formation and related disturbances, may be indicative of interspecific hybridity. The relatively low level of irregularity among the Alaskan *B. pumpellianus* clones may be associated with the fact that these plants are from areas where opportunities for interspecific hybridization are limited. The samples of the three species are small, but the trends may be significant.

Elliott and Love (8) were undoubtedly correct in emphasizing that a rather high degree of meiotic irregularity may be tolerated under natural conditions in a long-lived, cross-pollinated species such as *B. inermis*, which can spread aggressively by rhizomatous growth. However, the extent of aneuploidy and degree of meiotic irregularity reported in recent studies could be partly accounted for by lack of control over parentage of material used in these studies.

## Acknowledgments

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# RIBONUCLEASE ACTIVITY IN RUSTED WHEAT LEAVES[1]

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## Abstract

Extracts from primary leaves of Lee wheat were prepared at various days following inoculation with races of leaf rust and tested for ribonuclease (RNase) activity. As early as 24 hours after inoculation there was a marked increase in the specific activity of the enzyme in extracts of rusted host tissues. A further increase in activity was observed during later stages of infection, with the susceptible and resistant reacting tissue differing only in the degree of their response. Extracts from noninoculated control leaves exhibited a constant RNase activity throughout the period of observation. The germination medium and extracts from germinating uredospores contained comparatively little RNase activity. No direct evidence was obtained either for the possible release of the enzyme from particulate cellular fractions of the host tissue as a result of infection or for the removal of an RNase inhibitor in the host tissue responding to infection.

## Introduction

Histological (14) and biochemical (15) investigations on rust-infected leaves have demonstrated changes in the amount, composition, and distribution of ribonucleic acid (RNA) in parasitized tissues. During the initial stages of development of the parasite within the host tissues $P^{32}$-labelled orthophosphate (17) and glucose-$C^{14}$ (unpublished results) are incorporated into the RNA to a considerably larger extent than in uninoculated tissue. Microautoradiographic studies showed that the increased incorporation of RNA precursors occurs preferentially in uninvaded host tissue surrounding the parasitized areas of the leaf (unpublished results). As far as the authors are aware, no information is available on the extent to which RNA is degraded during the course of infection. This paper gives the determinations of RNase activity in extracts from rust spores and rusted and healthy wheat leaves.

## Materials and Methods

Wheat plants (*Triticum aestivum* L., var. Lee) were heavily inoculated at the seedling stage with uredospores of pure races of *Puccinia recondita* Rob. ex Desm. Races 15 and 1 were used for most inoculations, the former giving a susceptible reaction on Lee wheat, the latter producing a resistant one. In later experiments a virulent culture of race 126 was also used. Primary leaves were harvested at various times after inoculation, weighed, ground with solid $CO_2$ in a mortar at $+2°$ C, and extracted with four times their weight of 0.05 *M* acetate buffer (pH 7.0) containing 40 g "Carbowax 4000" per 100 ml of solution (6). After the suspension had been passed through four layers of cheesecloth, it was centrifuged (15 minutes at 35,000 × *g*). The pellet, which contained subcellular particulate material and the protein precipitated by "Carbowax 4000", was washed three times with the extraction medium,

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then resuspended in 0.05 *M* acetate buffer (pH 6.0). In later experiments this suspension was subjected to a fractionated centrifugation to obtain preparations of various particle size (see below). For most experiments, however, the combined particulate material was removed by centrifugation (105,000 × *g*, 60 minutes) and the resulting supernatant liquid assayed for RNase activity ("soluble RNase").

RNase activity was determined according to the method of McDonald (13). In most cases the assay system contained 4 mg of substrate (wheat RNA) and 0.05 *M* acetate buffer (pH 6.0) in a total volume of 0.8 ml. Following incubation (45 minutes, 37° C) the reaction was stopped with 0.8 ml of MacFayden's reagent (0.25% uranylacetate in 2.5% aqueous trichloroacetic acid). In blanks, MacFayden's reagent was added at zero time to the incubation mixture. After chilling and removal of the precipitate, suitable dilutions of the clear solution were prepared and their absorbencies at 260 mμ were determined spectrophotometrically (1-cm quartz cells; Beckman DK2). One enzyme unit was defined as that amount which, under the above conditions, caused a change of 0.005 units in optical density.

The amount of protein in solution was calculated from the optical density of the enzyme preparations at 260 mμ and 280 mμ (1). The isolated enzyme preparations retained their full activity when kept for 23 hours under refrigeration. However, storage for 22 days at −22° C resulted in a 50% loss of activity. All preparations were therefore assayed shortly after isolation. The optical density of the assay solutions increased linearly with increasing amounts of enzyme preparation in the incubation mixture, demonstrating that the amount of enzyme preparation was, under our conditions, the limiting factor in the reaction. All enzyme assays reported in this paper give, therefore, a measure of the amount of enzyme available for the reaction. Attempts to demonstrate RNase inhibitors in the extracts were unsuccessful. Low substrate concentrations (up to 0.5 mg per assay) increased RNase activity linearly; substrate concentrations above 4 mg RNA per assay sample resulted in gradual inhibition (9). For most assays wheat RNA, which had been isolated and purified (17) from mature primary leaves of a commercial variety, was used. It was found to be a better substrate for wheat RNase than a similarly prepared RNA from yeast.

## Results and Discussion

The curves in Fig. 1 show the drifts of specific activity of soluble RNase from non-infected and rusted wheat leaves, exhibiting either resistant or susceptible reactions, which were harvested at various times after inoculation. Extracts from uninfected control leaves exhibited a remarkable uniformity in their RNase content during the period of observation. In inoculated tissues, however, the specific activities of the RNase preparations rose to 100% over control levels within 24 hours after inoculation and amounted to approximately 300% in the susceptible reacting tissue at 6 days after inoculation (erumpent pustules). This postinfectional increase is plotted against time after inoculation in Fig. 1, and follows a bimodal curve in both the resistant and the susceptible reactions with the two reactions differing only in degree.

The bimodal characteristic of this curve may reflect different phases of rust development, since the drop in activity at 3 days after inoculation coincides with flecking. That the increase in specific activity from inoculated plants cannot be attributed to an equivalent drop in the protein content of these extracts is illustrated in Table I, which lists RNase activity and protein content of the preparations. The reproducibility of specific activity values was found to be satisfactory within individual experiments (see uninoculated controls, Fig. 1). A considerable amount of variation, however, was detected when extracts from plant material of different experimental runs were compared. Environmental conditions, under which the plants were grown, affected the specific activity of the extracts to a large extent (Table II). Other variable factors, including inoculation density and the extent to which the tissue was disintegrated upon homogenization, undoubtedly contributed to this variation.

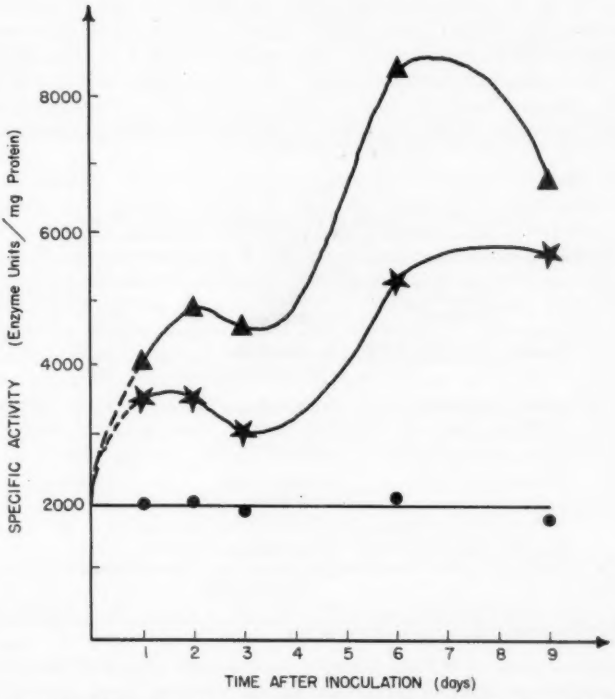


FIG. 1. Soluble RNase. Specific activity of extracts from primary leaves of Lee wheat at various times after inoculation with leaf rust.

● ● ●, uninoculated controls; ▲ ▲ ▲, inoculated with race 15 (susceptible reaction); ★ ★ ★, inoculated with race 1 (resistant reaction).

Rust development: fleck stage at 3 to 4 days, initial sporulation at 5 to 6 days after inoculation.

TABLE I

Ribonuclease activity and protein content of extracts from rusted primary leaves of Lee wheat

| Source | Days after inoculation | RNase activity of extract (enzyme units/ml) | Protein content of extract (mg/ml) |
|---|---|---|---|
| Uninoculated controls | 1 | 425 | 0.210 |
| | 2 | 415 | 0.205 |
| | 3 | 400 | 0.205 |
| | 6 | 400 | 0.195 |
| | 9 | 290 | 0.155 |
| Inoculated with race 15 (susceptible reaction) | 1 | 765 | 0.190 |
| | 2 | 895 | 0.180 |
| | 3 | 700 | 0.150 |
| | 6 | 1750 | 0.210 |
| | 9 | 1970 | 0.290 |
| Inoculated with race 1 (resistant reaction) | 1 | 750 | 0.210 |
| | 2 | 865 | 0.240 |
| | 3 | 750 | 0.240 |
| | 6 | 1350 | 0.250 |
| | 9 | 1175 | 0.205 |

NOTE: Extracts (final volume 13.5 ml) obtained from 5 to 8 g of leaf material. Rust development: fleck stage at 3 to 4 days, initial sporulation at 5 to 6 days after inoculation.

TABLE II

Comparison of RNase activities of extracts from wheat leaves of four independent experimental runs

| Experiment No. | Growth conditions | Sample* | Enzyme units/mg protein | % of uninoculated control |
|---|---|---|---|---|
| I | Growth chambers, 20° C | Uninoculated | 1276 | — |
| | | Uninoculated | 1442 | — |
| II | Growth chambers, 28° C | Uninoculated | 1548 | — |
| | | Inoculated with race 15 | 7183 | 464 |
| III | Greenhouse | Uninoculated | 2024 | — |
| | | Inoculated with race 15 | 4972 | 246 |
| IV | Growth chambers, 20° C | Uninoculated | 2045 | — |
| | | Inoculated with race 126 | 3087 | 151 |

*The leaves of experiments II and III were harvested at 2 days, those of experiment IV at 5 days after inoculation. Inoculation density in experiment IV was exceptionally low.

The large increase of RNase activity during later stages of the susceptible reaction cannot be due to extraction of the enzyme from attached fungal spores, since the isolation procedure used for extracting the plant material failed to disintegrate uredospores and did not yield detectable amounts of RNase from spore material treated in this manner. The possibility remains, however, that contents of early infection structures and of fungal mycelium within the host contribute to the postinfectional increase of soluble RNase.

Attempts were therefore carried out to estimate the contribution of fungal material to the increased RNase content of the infected host by determining the activity of the enzyme from exhaustively extracted uredospores germinating in vitro.

The uredospores (1.3 g, mixed races of *P. recondita*) were allowed to germinate for 4 hours on 0.005 *M* acetate buffer (pH 6.5) in large stainless steel sinks. To aid the germination, a $5 \times 10^{-4}$ *M* pelargonic aldehyde (5) solution was introduced on filter paper strips into the moisture-saturated atmosphere. Microscopical observation revealed no evidence of ruptured germ tubes at the end of the germination period, when approximately 80% of the spores had germinated. The germination medium (approximately 8 liters, now pH 6.0) was separated from the spore material by filtration.

The germinated spores were extracted for 9½ hours at +2° C in a ball mill with 0.05 *M* acetate (pH 6.5) containing 0.45 *M* sucrose, after which the crude debris was removed by centrifugation (1000 × *g*, 10 minutes). The supernatant liquid of a further centrifugation (105,000 × *g*, 60 minutes) was dialyzed against 0.05 *M* acetate (pH 6.0) and used for the determination of soluble RNase; the sedimented particulate material obtained from the high-speed centrifugation was resuspended in, and dialyzed against, the same buffer, and used for the assay of bound RNase after incubation in 6 *M* urea (see below).

The germination medium was concentrated *in vacuo* 200-fold (+35° C) with stepwise dialysis (at pH 6.0), and the molarity of the acetate buffer was kept at ≦0.05 at all times. At a final dialysis of the concentrated preparation (0.05 *M* acetate, pH 6.0), 12.5% glucose was included in the dialysis medium, for stabilization of the relatively dilute protein solution (7). This preparation served for the assay of RNase excreted from the spore material during germination.

From Table III it is seen that, under conditions designed to extract RNase from spore material, over 10,000 units of the enzyme were recovered from 1 g (fresh weight) of germinating uredospores; approximately one-quarter of the enzyme activity was present in the germination medium. This amount of RNase can hardly account for the postinfectional increase of the enzyme in

TABLE III

Endogenous and excreted RNase recovered from germinating uredospores (1 g) of leaf rust

| Fraction* | Enzyme units | mg protein | Enzyme units/ mg protein |
|---|---|---|---|
| Spore extract | | | |
| Combined particulate fractions† | 4423 | — | — |
| Supernatant liquid‡ | 3700 | 9.8 | 379 |
| Germination medium | 2356 | 24.0 | 97 |
| Total | 10479 | — | — |

*For preparation and assay of fractions see text.
†Containing all material sedimenting at 105,000 × *g* for 60 minutes. Particles preincubated for 10 minutes in 6 *M* urea before assay.
‡Supernatant liquid of centrifugation at 105,000 × *g* for 60 minutes.

infected leaves, since very much less spore material, certainly not exceeding 10 mg, was used for the inoculation of the leaf material. The possible contribution of fungal material to the increased amount of RNase in the host/parasite complex can still not be ruled out entirely, since it is conceivable that germinating spores produce sufficient amounts of RNase only when germinating on the host; alternatively, early vegetative structures of the fungus in the host tissue may account for the "excessive" amount of RNase observed soon after inoculation. Table III also shows that relatively large amounts of protein, other than RNase, are released into the germination medium. This is in agreement with similar findings by Shu and Tanner (20).

A further possibility for the postinfectional increase of the enzyme is the possible occurrence of a RNase inhibitor in uninoculated leaves remaining in the preparations. Removal of this inhibition during the course of infection would result in an increase of enzyme activity. No direct evidence of an inhibitor was obtained in our RNase preparations; extracts from healthy leaves when mixed with those from inoculated leaves had no effect on enzyme activity. However, this procedure would not demonstrate the presence of an inactivating agent similar to the proteinaceous compound from rat liver (18). A soluble, dialyzable RNase inhibitor from higher plants was described by Bernheimer and Steele (3). If a similar inhibitor occurs in wheat leaves, it probably would have been removed by the current extraction procedure and thus could not account for the present results.

The observed increase of soluble RNase in the host tissue following infection could conceivably arise through liberation of the enzyme, either in vivo or during the course of extraction, from other structural components of host cells into the soluble fraction. A postinfectional release of RNase from particulate material would thus result in an increase in the specific activity of the enzyme in the soluble fraction without a net increase of over-all enzyme content in the tissue. An attempt was therefore made to determine the relative amount of RNase in subfractions of tissue homogenates from non-infected and rusted leaf material. After removal of "Carbowax"-soluble material (see Materials and Methods) the preparations were subjected to a fractionated centrifugation (1000 × *g*, 10 minutes; 15,000 × *g*, 30 minutes; 105,000 × *g*, 60 minutes), which yielded pellets that were arbitrarily termed "chloroplast", "mitochondria", and "microsome" fractions, and which produced a final supernatant liquid comparable to the soluble RNase fraction used above. The cell-wall material was not tested for RNase activity. The designations of particulate fractions are arbitrary and do not represent homogeneous preparations of these fractions. Some preliminary experiments showed that the particulate material possessed considerable RNase activity.

It has been demonstrated that urea, ethylenediamine tetraacetic acid, and other compounds aid in the release of RNase, other enzyme proteins, as well as RNA from microsomal particles of bacteria (4, 8, 22), rat tissue (19, 21), pea seedlings (11, 12), and wheat roots (10). The effect of high concentrations of urea on the release of RNase activity from isolated particulate fractions of wheat leaves is briefly summarized in Table IV. Preincubation in 6 *M* urea resulted in a considerable release of RNase activity, amounting to 4-, 10-, and 20-fold increases in the chloroplast, mitochondrial, and micro-

somal fractions, respectively. Addition of exogenous substrate proved inhibitory to all but the chloroplast fractions. This can be attributed to the relatively high RNA content of the mitochondrial and microsomal fractions; addition of further substrate presumably causes inhibition of the enzyme observed at excessive substrate concentrations (9). A brief 10-minute treatment at room temperature in 6 *M* urea resulted in a maximum release of the enzyme from the particulate fractions. No effort was made to determine the extent to which particle-bound RNase is released by this treatment, since the various preparations were treated similarly and were to be compared on a relative basis only.

TABLE IV

Effect of urea on RNase activity of isolated cellular fractions

| Assay system* | Enzyme units per fraction† from 1 g fresh weight of leaves | | |
|---|---|---|---|
| | Chloroplasts | Mitochondria | Microsomes |
| Preincubated in buffer: | | | |
| Complete | 2303 | 235 | 94 |
| No substrate | 425 | 423 | 282 |
| Preincubated in 6 *M* urea: | | | |
| Complete | 8836 | 2491 | 2350 |
| No substrate | 3149 | 5499 | 5123 |

*Complete system contained: 4 mg wheat RNA, particulate fraction (preincubated for 10 minutes at room temperature in buffer or 6 *M* urea), 0.05 *M* acetate, pH 6.0; total volume 0.8 ml.

†Designations of fractions are arbitrary and do not represent homogeneous preparations of these fractions. The chloroplast fraction contains all material sedimenting at 1,000 × *g* (10 minutes); the mitochondrial and microsomal fractions include all material sedimenting between 1,000 × *g* (10 minutes) and 15,000 × *g* (30 minutes) and between 15,000 × *g* (30 minutes) and 105,000 × *g* (60 minutes) respectively.

A comparison of the enzyme activity in fractionated preparations from noninoculated and rusted leaf material (Fig. 2) showed that the postinfectional increase of the enzyme is predominantly due to an increase in the soluble fraction, although most of the activity is contained in the particulate material. If a release of RNase under the influence of the infection had occurred from any of the particle fractions, a comparable decrease of activity in the particulate material would be expected. This is clearly not the case. Although the over-all activity of particulate fractions is nearly the same in noninoculated and rusted leaf material, some differences can be observed when individual fractions from these tissues are compared. Thus, the mitochondrial preparation from rusted leaves contained 158% of the RNase activity recovered in a similar fraction from noninoculated tissue; conversely, only 94% of the enzyme activity was recovered from the chloroplast fraction following inoculation. These differences may be artifacts of isolation rather than a consequence of infection since the disintegration of tissue samples was not standardized and a larger proportion of chloroplast fragments may have contaminated the "mitochondrial" fraction from rusted plant material (see Fig. 2). The interpretation of these results is further complicated since no information is available on the percentage enzyme release from the various cellular particles following treatment with urea. RNase may, for instance, be more effectively released from particles of inoculated than from those of nuinoculated plants.

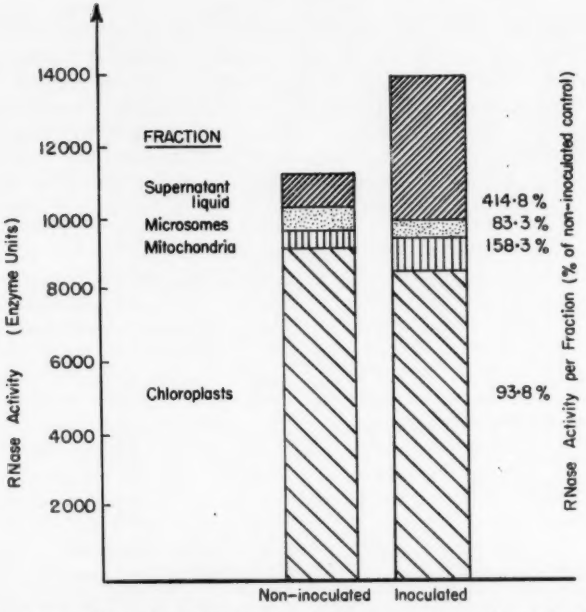


FIG. 2. RNase activity of cellular fractions isolated from 1 g fresh weight of primary leaves of Lee wheat, harvested 2 days after inoculation with leaf rust (race 15).
Assay system contained: aliquot of cellular fraction, 4 mg wheat RNA, 0.05 *M* acetate (ph 6.0); total volume 0.8 ml. All particulate fractions preincubated in 6 *M* urea.
Designations of fractions are arbitrary and do not represent homogeneous preparations of these fractions (see legend Table IV).

Since our results do not indicate a release of RNase from cellular particles following infection, other possibilities for the postinfectional increase in soluble RNase must be considered. *De novo* synthesis is such a possibility and it would, in our experiments, remain undetected if the newly synthesized enzyme protein were released from its synthetic site (e.g., microsomes) immediately after its formation. Preliminary attempts to induce RNase formation in uninoculated leaves treated with extracts from rusted leaves were unsuccessful.

All our experiments show that in extracts from infected wheat leaves, whether they are resistant or susceptible, there is a consistent increase in soluble RNase. Perhaps most significantly, this increase takes place at a very early stage during disease development, and amounts to approximately 100% over control values during the first 24 hours after inoculation. Conceivably, this may therefore reflect a primary interaction between host and parasite. It is of interest to note that all other previously recorded physiological and biochemical changes in the host/parasite complex occur much later in the development of the parasite. Until changes in RNase content are demonstrated in vivo it is not possible to determine whether they are of any significance in

the development of the disease. Further information is also needed on the mechanism which leads to the increase in RNase activity in the soluble fraction. In the present study no attempt was made to purify the enzyme extract or determine whether the activity is due to RNase and (or) some other less specific phosphodiesterase. The possibility must therefore be kept in mind that our preparations may contain more than one RNA-degrading enzyme, similar to those reported by Matsushita and Ibuki (11), with perhaps one originating from the parasite and accounting for the additional activity following inoculation. In addition, host RNA and rust RNA may not be equally susceptible or equally accessible to degradation by the enzyme(s). If a postinfectional increase of RNase activity can be demonstrated in vivo, it would then be of particular interest to know where in the parasitized tissue it occurs. Such information is difficult to obtain with the current biochemical approach. Clearly, a histochemical test for RNase activity is needed.

The increased RNase activity in the wheat/rust system parallels Reddi's (16) results on the increased activity of this enzyme in tobacco leaves following infection with TMV, where the enzyme activity undoubtedly originates from the host. This author suggests that host RNase may be implicated in the synthesis of viral RNA. A synthetic function of RNase during the germination of peas was recently proposed by Barker and Douglas (2). Our results do not support any such speculation on the role of RNase in infected tissues.

Previous investigations showed that rust infection results in a net increase of RNA (15), and a higher incorporation rate of precursors into the RNA (17) of the host/parasite complex. An increased RNase activity in vivo would therefore suggest an accelerated turnover rate of the substrate. This view is in agreement with the fact that rusted tissues generally exhibit a higher metabolic rate. Our in vitro studies indicate that such changes may occur very shortly after infection.

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# GENETICS OF PHYTOPATHOGENIC FUNGI

## VI. HETEROCARYONS INVOLVING GIBBERELLA FUJIKUROI AND FORMAE OF FUSARIUM OXYSPORUM[1]

T. S. Dhillon, E. D. Garber, and Ellen G. Wyttenbach


## Abstract

Heterocaryons involving nutritionally deficient mutant strains of *Gibberella fujikuroi* and mutant strains of this species and of four formae of *Fusarium oxysporum* were obtained. It was not possible to get heterocaryons of *G. fujikuroi* and *F. oxysporum* f. *pisi* until the latter mutant strain was adapted to grow on minimal medium. Spores from heterocaryons had either the genotype of only one component strain or of either component strain, depending on the heterocaryon. It was possible to alter nuclear ratios by growing certain heterocaryons on minimal medium supplemented with the compound(s) required by one or the other component strain. Sectors from certain heterocaryons grown on potato dextrose agar were homocaryotic for the nutritional requirement(s) of one component strain. Heterocaryosis as a taxonomic tool and the difficulties involved in demonstrating the parasexual cycle in the genus *Fusarium* are discussed.


## Introduction

Nutritionally deficient mutant strains have made it possible to synthesize heterocaryons in an almost routine fashion. Information, however, on the behavior of heterocaryons in filamentous fungi has been obtained mostly from *Neurospora* and *Aspergillus*. It is becoming increasingly clear that heterocaryons of other fungal genera may not conform in many respects with those of *Neurospora* and *Aspergillus*.

Taschdjian and Muskatblitt (8) consider hyphal fusion or anastomosis in imperfect fungi as a possible criterion to recognize species, by assuming that hyphal fusion generally occurs between members of the same species. A visual demonstration of fusion, however, may be difficult or subjective. The isolation of a heterocaryon involving mutant strains with different nutritional requirements provides an acceptable basis for assuming that hyphal fusion had occurred. Gibson and Griffin (4), however, have provided evidence to warrant caution in accepting growth on minimal medium as the sole criterion for identifying a heterocaryon involving nutritionally deficient mutant strains.

The imperfect genus *Fusarium* is extremely variable. Species have been identified on the basis of certain morphological attributes; genera have been recognized when isolants yielded the perfect stage. The hierarchy of taxa within a species also raises a question of relationship. A category, *forma specialis*, has been recognized in *F. oxysporum* to accommodate differences in host specificity. For example, the isolant responsible for *Fusarium* wilt of tomato is identified as *F. oxysporum* f. *lycopersici*. A forma may also include physiological races distinguishable on the basis of their virulence for different varieties of the host species.

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This paper presents data on heterocaryons involving nutritionally deficient mutant strains of *Gibberella fujikuroi* and of heterocaryons involving mutant strains of this species and formae of *F. oxysporum*.

## Materials and Methods

Strain NRRL 2284 of *G. fujikuroi* (Saw.) Wr. (*Fusarium moniliforme* Sheld.) was obtained from Dr. C. L. Haseltine; the origin of the four formae of *F. oxysporum* Schl. has been presented by Garber *et al.* (3).

Except for *f. conglutinans*, growth and sporulation of the strains and mutants were excellent on a complex medium containing potato dextrose agar (Difco), supplemented with 0.2% yeast extract (Difco), and on a synthetic or minimal medium used for growing *Chlorella* (1), supplemented with 2% glucose.

Cultural conditions and methods for harvesting spores, for obtaining and characterizing nutritionally deficient mutants, and for making heterocaryons have been presented in detail by Tuveson and Garber (9, 10).

Component strains of each heterocaryon were grown as controls in all experiments involving the growth of spores or mycelial fragments on complex or minimal media. Spores were transferred with a multineedle inoculating device, described by Pontecorvo (6), to determine the nutritional requirements of colonies. The concentration of each supplement added to minimal medium is presented in Table I.

TABLE I

Supplementation level of nutrients

| Nutrients | Mg/ml of minimal medium |
|---|---|
| DL-Tryptophan (try) | 0.25 |
| Adenine (ade) | 0.002 |
| Para-aminobenzoic acid (pab) | 0.001 |
| Inositol (ino) | 0.001 |
| Nicotinic acid (nic) | 0.00025 |
| Biotin (bio) | 0.00010 |

## Results

*Mutant Strains*

The nutritional requirements, color, and morphology of the mutant strains of *G. fujikuroi* and of the formae of *F. oxysporum* appear in Table II. Resistance to acriflavine (0.005%) in complex medium was a valuable marker found only in f. *pisi*. The color markers were noted for colonies grown on complex medium. The carmine color of the f. *pisi* mutant was strikingly different from the red color of other mutant strains; obvious red threads were noted only in the carmine colonies. The degree of fluffiness in different mutant strains reflected the density and height of the short, aerial hyphae. The diffuse, transparent colony of adenine-requiring mutants on minimal medium supplemented with this compound distinguished mutants with this requirement from other nutritionally deficient mutants grown on minimal medium supplemented with the appropriate compound.

TABLE II

Mutant strains of *Gibberella fujikuroi* and of formae of *Fusarium oxysporum*

| Strain | Genotype | Description of colonies on: Potato dextrose agar | Supplemented minimal agar |
|---|---|---|---|
| Gf-1 | pab, red | Red, very fluffy | White, fluffy |
| Gf-2 | pab, whi, but | White, fluffy; button colony | White, fluffy |
| Gf-3 | bio, red | Red, fluffy | White, fluffy |
| Gf-4 | ade, red | Red, fluffy; obvious white halo | Diffuse, transparent |
| Gf-5 | pab, ade, red | Red, fluffy; white halo | Diffuse, transparent |
| Gf-6 | pab, bio, red | Red, flat | White, fluffy |
| Fn-7 | try, whi | White, extremely fluffy; reddish with prolonged incubation | White, very fluffy |
| Fc-8 | ade, ora | Orange, fluffy | Orange, diffuse, transparent |
| Fp-9 | ino, car | Carmine, felty; red threads | Red, felty; slow-growing |
| Fl-10 | nic, ora | Orange, fluffy | Orange, very fluffy |

NOTE: Gf = *G. fujikuroi*; Fn = *niveum*; Fc = *conglutinans*; Fp = *pisi*; Fl = *lycopersici*.

*Origin of Heterocaryons*

Spores from the component strains of each heterocaryon (HET) were mixed to give a turbid suspension and a drop added to the surface of plates of minimal agar; spores from each component strain were seeded as controls. The component strains displayed no significant growth within the period of incubation required to detect vigorous outgrowths of mycelium from the mixed-spore suspensions. The significance of the long incubation period needed to observe mycelial outgrowths from the spore mixtures involving f. *pisi* will be presented later. The mycelial outgrowths were considered to be heterocaryotic hyphae; transfers were made to complex and minimal media for further analysis.

*Description of Heterocaryons*

A description of heterocaryons grown on complex and minimal media is presented in Table III. Except for the occurrence of extremely fluffy sectors in HET XIII, heterocaryons involving only mutant strains of *G. fujikuroi* were usually indistinguishable from colonies of the component strains on complex medium. Colonies of HET XXII which involved a button and a non-button strain resembled the latter component.

Except for HET XVIII and HET XIX, heterocaryons involving *G. fujikuroi* and formae of *F. oxysporum* did not resemble either component strain on complex or supplemented minimal media. The exceptional heterocaryons resembled *G. fujikuroi*. It should be noted that f. *conglutinans* grew relatively slowly on both media. Garber *et al.* (3) found that heterocaryons involving f. *conglutinans* and another forma usually resembled the latter component.

*Conidia from Heterocaryons of G. Fujikuroi*

Heterocaryons were grown on complex and minimal media for 7–9 days; the harvested spores were then plated on these media. Spores from HET XIV yielded colonies on minimal medium and these were termed "prototrophic"; spores from the other heterocaryons yielded colonies only on complex medium and had the genotype of one component strain (Table IV).

The growth of the "prototrophic" colonies was enhanced on minimal medium

TABLE III

Colony characteristics of heterocaryons on complex and minimal media

| Hetero-caryon | Component strains | Potato dextrose agar 7-day-old colony | Minimal agar 15-day-old colony |
|---|---|---|---|
| XII | Gf-1, Gf-3 | Red, very fluffy | White, fluffy |
| XIII | Gf-1, Gf-4 | Red, very fluffy; sectors after 3–4 days | White; fluffy; red patches on bottom surface |
| XIV | Gf-3, Gf-4 | Red, very fluffy | White, fluffy |
| XX | Gf-4, Gf-6 | Red, fluffy | White, fluffy; slow-growing |
| XXII | Gf-2, Gf-4 | Red, fluffy; conspicuous white halo | White, fluffy |
| XV | Gf-5, Fp-9 | Red-carmine, fluffy; white center | White, fluffy; aerial hyphae; periphery becoming light purple with incubation |
| XXI | Gf-4, Fp-9 | Red, fluffy; carmine sectors with red threads after 5–6 days | White, fluffy; purple patches in center and purple ring at periphery; aerial hyphae |
| XVI | Gf-5, Fl-10 | Red threads on orange background, fluffy | Orange, fluffy; deep orange bottom surface; brown-yellow medium adjacent to colony |
| XVII | Gf-5, Fn-7 | Gray streaks on white background; red or orange sectors after 4–5 days | White, extremely fluffy |
| XVIII | Gf-5, Fc-8 | Red, fluffy | White, fluffy |
| XIX | Gf-6, Fc-8 | Red, fluffy | White, fluffy; slow-growing |

supplemented with biotin, a requirement of one of the component strains of HET XIV. It was possible to obtain spores from the colonies on minimal medium which again yielded colonies on this medium. This procedure was repeated for five successive transfers.

Spores of HET XII and HET XIV grown on minimal or complex media had the genotype of only one component strain. One spore from HET XIII grown on minimal medium required adenine, and the remaining spores, para-aminobenzoic acid. Spores from HET XX and XXII grown on minimal medium had the genotype of either component strain.

*Conidia from Heterocaryons involving G. fujikuroi and Formae of F. oxysporum*

Heterocaryons were grown on complex and minimal media for 7–9 days; the harvested spores were then plated on these media and the resulting colonies were tested for their nutritional requirements (Table IV). Spores from the heterocaryons involving f. *pisi* yielded colonies on minimal medium; growth of these colonies was enhanced when the medium was supplemented with inositol. It should be noted that "prototrophic" colonies were also obtained from HET XVII but these were later identified as heterocaryons, presumably from mycelial fragments.

Spores from the heterocaryons grown on complex medium included either nuclei from only one component strain or nuclei from either component strain, depending on the particular heterocaryon. Except for HET XVI, the genotypes of spores from heterocaryons grown on minimal medium were the same as those of spores from heterocaryons grown on complex medium. Whereas spores from HET XVI on complex medium included nuclei from only one component strain, spores from this heterocaryon on minimal medium included nuclei from either component strain.

*"Prototrophy" and Adaptation*

As previously mentioned, mixed-spore suspensions of *G. fujikuroi* and f. *pisi* required a longer incubation to obtain mycelial outgrowths than suspensions involving other formae. To get heterocaryons of *G. fujikuroi* and f. *pisi*, mixed-spore suspensions were seeded on complex medium; the resulting mycelium was shredded and washed, and the small pieces placed on minimal medium (10). No obvious growth was observed during the first week of incubation; dark red patches appeared during the second week and increased in size during the third week; fluffy, white mycelial outgrowths were noted during the fourth week. Mycelium was transferred to minimal medium where growth was prompt and rapid. The harvested spores grew on minimal and complex media containing acriflavine; growth was enhanced on minimal medium containing inositol. Spores from these presumptive heterocaryons did not differ from those obtained from heterocaryons synthesized directly from spore mixtures. Only one type of colony was obtained from HET XV; two morphologically different colonies were observed among the "prototrophs" on minimal medium from HET XXI. One type was extremely fluffy and grew rapidly (Figs. 1, 2); the other type was slightly fluffy and grew slowly (Figs. 3, 4). These differences were maintained during five transfers, using spores, on minimal medium.

Since it was possible that adaptation of the f. *pisi* strain, and not heterocaryosis may have been responsible for the appearance of mycelial outgrowths after an extended incubation of mixed-spore suspensions, mycelium of the f. *pisi* component (strain 9) was transferred to minimal medium and incubated. No growth was observed during the first week (Fig. 7); slight growth was seen on the surface of the transfer during the next 2 weeks; a rapid, spreading growth, which eventually covered the surface of the plate, was noted during the fourth week. Spores were then harvested and plated on minimal medium where they yielded colonies (Figs. 5, 6); growth was enhanced on minimal medium containing inositol. Using spores from colonies, five serial transfers on minimal medium gave the same results. On the other hand, spores from strain 9 grown on complex medium did not yield colonies on minimal medium (Fig. 8).

Although spores from the heterocaryons and from adapted strain 9 were able to grow on minimal medium, it was possible to distinguish between these colonies. On complex medium, "prototrophic" colonies from the heterocaryons were always extremely fluffy and "prototrophic" colonies from adapted strain 9 were slightly fluffy. Colonies from the unadapted strain 9 were felty on this medium. The differences were sufficiently marked to permit a positive identification of the source of each colony.

*Conidia from Heterocaryons Grown on Supplemented Minimal Medium*

Tuveson and Garber (11) were able to manipulate nuclear ratios in certain heterocaryons involving nutritionally deficient mutant strains of *F. oxysporum* f. *pisi* by growing the heterocaryons on minimal medium supplemented with graded concentrations of the compounds required by the "recessive" nuclei. One heterocaryon (HET XXII) involving mutant strains of *G. fujikuroi* and three heterocaryons involving this species and f. *lycopersici* (HET XVI) or f. *pisi* (HET XV, HET XXI) were grown on supplemented minimal medium

TABLE IV

Nutritional requirements and color of colonies from spores harvested from heterocaryons

| Hetero-caryon | No. of spores | Heterocaryon grown on potato dextrose agar | No. of spores | Heterocaryon grown on minimal agar |
|---|---|---|---|---|
| XII | 102 | 102—pab, red | 52 | 52—pab, red |
| XIII | 104 | 104—pab, red | 52 | 51—pab, red; 1-ade, red |
| XIV | 104 | 104—"prototrophic", red | 52 | 52—"prototrophic", red |
| XX | 51 | 51—pab, bio, red | 102 | 91—pab, bio, red; 11—ade, red |
| XXII | 77 | 77—ade, red | 121 | 96—ade, red; 25—pab, white |
| XV | 76 | 76—"prototrophic", carmine | 103 | 103—"prototrophic", carmine |
| XXI | 52 | 52—"prototrophic", carmine | 52 | 52—"prototrophic", carmine |
| XVI | 104 | 104—nic, orange | 104 | 98—nic, orange; 6—pab, ade, red |
| XVII | 101 | 18—pab, ade, red; 76—try, white; 3—"prototrophic" white | 104 | 59—pab, ade, red; 45—try, white |
| XVIII | 104 | 104—pab, red | 52 | 52—pab, red |
| XIX | 104 | 104—pab, bio red | 102 | 102—pab, bio, red |

TABLE V

Nutritional requirements of spores harvested from heterocaryons grown on supplemented minimal agar

| Heterocaryon | Supplement* | No. of spores | Observations |
|---|---|---|---|
| XXII | Adenine | 100 | 86—pab; 14—ade |
| | pab | 150 | 150—pab |
| XVI | 10 mg % ade | 243 | 68—pab, ade; 175—nic |
| | 20 mg % ade | 196 | 72—pab, ade; 124—nic |
| | 30 mg % ade | 231 | 69—pab, ade; 162—nic |
| | 40 mg % ade | 291 | 155—pab, ade; 136—nic |
| | 50 mg % ade | 52 | 16—pab, ade; 36—nic |
| XXI | Adenine | 52 | 52—"prototrophic" |
| | Inositol | 52 | 48—"prototrophic"; 4—ade |

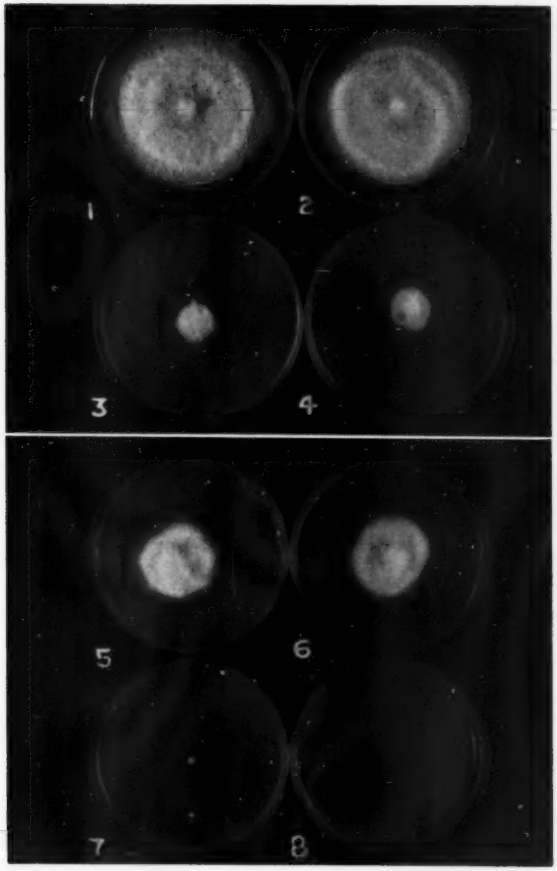
*See Table I for concentration of supplements; mg % = milligrams per 100 ml of medium.

and the harvested spores were tested for their nutritional requirements (Table V).

Except for HET XVI, the concentrations of the supplements have been presented in Table I. Approximately 14% of spores from HET XXII, grown on minimal medium containing adenine, required this compound; all spores from the heterocaryon, grown on minimal medium containing para-aminobenzoic acid, required this compound. These observations were correlated with the colonial morphology of the component strains: the *pab* mutants were buttons and the *ade* mutants, non-buttons. The frequency of spores requiring adenine was increased on minimal medium containing this compound compared with that of spores from the heterocaryon grown on minimal medium.

The results obtained with HET XXI were significant since it was possible that this heterocaryon involving f. *pisi* may have been merely an adapted strain 9. Colonies of this heterocaryon, grown on minimal medium containing inositol, yielded spores requiring adenine. Consequently, HET XXI included

PLATE I




FIGS. 1–8. Colonies on minimal medium from spores of heterocaryon XXI (*Gibberella fujikuroi* and *Fusarium oxysporum* f. *pisi*), of an adapted, inositol-requiring mutant strain of *F. oxysporum* f. *pisi*, and a mycelial transfer of the non-adapted, inositol-requiring mutant strain. Figs. 1, 2. Rapidly growing colonies from heterocaryon XXI. Figs. 3, 4. Slowly growing colonies from the same heterocaryon. Figs. 5, 6. Colonies from the adapted, inositol-requiring mutant strain. Fig. 7. Colony from mycelial transfer of non-adapted, inositol-requiring mutant strain. Fig. 8. Plate seeded with spores of the inositol-requiring mutant strain grown on potato-dextrose agar.

nuclei from the component strains and did not represent an adapted strain 9, the f. *pisi* component. Only "prototrophic" spores were obtained from HET XV, regardless of supplementation.

*Nutritional Requirements of Sectors*

Five heterocaryons exhibited obvious sectors or streaks on complex medium; no heterocaryon displayed either streaks or sectors on minimal medium (Table II). Sectors from HET XIII required para-aminobenzoic acid; sectors from HET XVII required either tryptophan or para-aminobenzoic acid plus adenine; sectors from HET XXI were "prototrophic." Sectors appeared in heterocaryons XX, XXII, XV, and XXI grown on minimal medium containing adenine, and, in one case, para-aminobenzoic acid. In all four heterocaryons, the sectors required the compound(s) used as supplements. These observations are summarized in Table VI.

TABLE VI

Nutritional requirements of sectors of heterocaryons

| Hetero-caryon | Potato dextrose agar. Sector required: | Heterocaryon grown on: Minimal medium | |
|---|---|---|---|
| | | Supplemented with: | Sector required: |
| XIII | pab | Not done | Not done |
| XX | Not done | Adenine | Adenine |
| XXII | Not done | Adenine | Adenine |
| XV | Not done | pab, ade | pab, ade |
| XVII | try or pab, ade | Not done | Not done |
| XXI | "Prototrophic" | Adenine | Adenine |

NOTE: pab = para-aminobenzoic acid; ade = adenine; try = tryptophan.

Sectoring was not observed in HET XVIII on complex or minimal media. Mycelial fragments were taken in sequence along the diameter of a colony on minimal medium and transferred to plates containing this medium either with or without adenine. Only one of six transfers grew better on the supplemented minimal medium. Spores from this colony were found to require para-aminobenzoic acid. Although it is not clear why these spores did not also require adenine, this observation indicated that sectoring may occur on minimal medium and may not be correlated with a morphological alteration.

## Discussion

Heterocaryons involving nutritionally deficient mutant strains (1) of *Gibberella fujikuroi*, (2) of physiological races of *Fusarium oxysporum* f. *pisi* (10), and (3) between *G. fujikuroi* and three formae of *F. oxysporum* have been obtained. The genotype of spores from these heterocaryons corresponded either with that of only one component strain or of either component strain, depending on the heterocaryon and on the medium. Furthermore, it was possible to alter nuclear ratios in certain heterocaryons in each of the three categories by growing the heterocaryons on minimal medium supplemented with compound(s) required by one or the other component strain.

Buxton (2) found perithecia in a nutritionally deficient mutant strain of an isolant identified as *F. oxysporum* f. *pisi*; the perithecia were identified as those

of the perfect species *Nectria haematococcus* Berk. Gordon (5) questioned the original identification and suggested that the isolant was *F. solani* f. *pisi* (Jones) S. & H. Since the culture of *F. oxysporum* f. *pisi* used in our experiments had been obtained from Dr. Buxton, it may be assumed that this material was *N. haematococcus*. The resistance of *F. oxysporum* f. *pisi* and of *N. haematococcus* (kindly supplied by Dr. Buxton) to concentrations of acriflavine inhibitory to the other formae of *F. oxysporum* lends support to this assumption (unpublished). Consequently, it may be assumed that *G. fujikuroi* and *N. haematococcus* have been involved in an "intergeneric" heterocaryon. Thus, it seems reasonable to assume that membership in the imperfect genus *Fusarium* may imply a closer relationship than membership in the perfect genera obtained from *Fusarium*.

Heterocaryosis and the isolation of a prototrophic, diploid, heterozygous spore from a heterocaryon involving nutritionally deficient mutant strains constitute the first and second steps in demonstrating the parasexual cycle in filamentous fungi (6). Since the parasexual cycle provides a method of investigating the genetics of pathogenicity in imperfect fungi, it is important to consider the possible origins of prototrophic colonies obtained by plating spore suspensions from heterocaryons. At least five different sources have been found: (1) heterocaryotic mycelial fragments (HET XVII, Table IV), (2) mutation from auxotrophy to prototrophy (7), (3) cytoplasmic mixing (3), (4) adaptation (HET XV, XXI, Table IV), and (5) diploidy (6). These diverse sources of "prototrophic colonies" provide an additional problem in screening spores from heterocaryons involving nutritionally deficient mutant strains, and consequently restrict the possibilities offered by the parasexual cycle for studying the genetics of pathogenicity. Nevertheless, it may be possible to use the parasexual cycle for such studies but only with the full knowledge that the individual steps in the cycle may differ significantly in each species of filamentous fungi, compared with *Aspergillus* (6).

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# STUDIES ON ASCOCHYTA IMPERFECTA PECK

## PARASITIC STRAINS AMONG FIFTY ISOLATES FROM CANADIAN ALFALFA SEED[1]

H. W. MEAD AND M. W. CORMACK


## Abstract

Fifty isolates of *Ascochyta imperfecta* from alfalfa seed grown in Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia produced varying degrees of infection on excised leaves of *Medicago falcata*, *Medicago sativa* (three strains), *Melilotus alba*, *Melilotus officinalis*, *Trifolium pratense*, *Lotus corniculatus*, *Pisum sativum*, and *Vicia faba*. *F* tests from analyses of variance of disease ratings showed that the differences in susceptibility between hosts and between strains of *Medicago* were highly significant. Among the 10 hosts and within the strains of *Medicago*, the interaction isolates × hosts was highly significant. Thus, specificity among the isolates was clearly demonstrated. This is interpreted to mean that parasitic strains existed among the 50 isolates.

*A. imperfecta* was isolated from 50 to 70% of the seed samples from each province. The heaviest infestation was on seed from Manitoba, where the weather at harvest time was wet and where the crop overwintered in the field.


## Introduction

Inoculation and cultural studies on *Ascochyta imperfecta* have shown that there are distinct differences between isolates. Edmunds and Hanson (2) demonstrated that there were highly significant differences in pathogenicity regarding host, location, and year of isolation. Kernkamp and Hemerick (4) found diverse cultural types among a large number of isolates. Representatives of these types appeared equally pathogenic on 69 different clones of alfalfa. Inoculation tests in the greenhouse also demonstrated that these isolates were pathogenic on 19 other species of legumes. Cormack (1) and Sprague (9) found that *Medicago sativa* was more susceptible to *A. imperfecta* than *Melilotus* and *Trifolium*. Toovey, Waterston, and Brooks (7) showed that *A. imperfecta* could attack *M. sativa* and *T. pratense* but not *Vicia faba* or *Pisum sativum*. Schenk and Gerdemann (8) showed that all of their isolates were more pathogenic on the hosts from which they were isolated than on others. Ellingboe and Kernkamp (3) grouped 112 isolates from alfalfa and red clover into seven rather distinct cultural groups; all of their isolates were pathogenic on alfalfa and red clover. None of the above studies demonstrated the existence of parasitic strains among isolates of *A. imperfecta*. The purpose of the present investigation was to determine the pathogenicity of isolates from Canadian alfalfa seed on 10 leguminous hosts, and to examine possible specific relationships of the isolates to these hosts.

## Materials and Methods

Sixty-four samples of alfalfa seed produced in 1958 were obtained from the laboratories of the Production and Marketing Branch, Canada Department of

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Contribution No. 82, Canada Department of Agriculture Research Station, Saskatoon, Sask.

Agriculture, in Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia. Two hundred seeds from each sample were placed on acidified potato lactose agar, without surface sterilization or washing, and were incubated at 24° C. Colonies of *A. imperfecta* were recognizable in 10 days. Isolates were transferred to potato lactose agar containing rose bengal and streptomycin to rid them of bacteria. Subisolates were then grown on acidified potato lactose agar in Petri dishes at 24° C. One isolate from each of the 50 seed lots in which the pathogen occurred was used in the inoculation trials. Inoculum was prepared by scraping the surface of 3-week-old cultures under sterile water, and screening the resultant spore suspensions through fine copper mesh. All spore suspensions were standardized as to density.

An excised leaf technique of inoculation, as described by Mead and Downey (5), was used in these experiments. Sixteen leaves from each of the 10 hosts, placed in four Petri dishes (four replicates), were inoculated with spore suspensions from the 50 isolates chosen for these experiments. The extent of lesioning on the leaves was rated by means of a disease scale, 0–5, where 5 meant a dead leaf (Fig. 1).

The following hosts were inoculated: alfalfa, *Medicago sativa* L. (Grimm and two polycross lines), and *M. falcata* L., red clover, *Trifolium pratense* L., sweet clover, *Melilotus alba* Desv., and *M. officinalis* L. (Lamb); birds-foot trefoil, *Lotus corniculatus* L., broad bean, *Vicia faba* L., and pea, *Pisum sativum* L.

## Results

*Isolations from Alfalfa Seed*

Colonies of *A. imperfecta* were obtained from alfalfa seed produced in all five provinces, but the percentage of seeds yielding the fungus varied (Table I). It was highest in Manitoba where adverse weather conditions prevented threshing of the alfalfa until the following spring. Fifty of the 64 samples of seed were infected with the pathogen.

All of the isolates obtained on potato lactose agar were olive-green in color. They differed in amount of aerial mycelium, but no significant difference in size and septation of spores could be demonstrated. Isolates differed in ability to form pycnidia on the agar, and also on the inoculated leaves. Ten of the isolates produced mature pycnidia on leaves of *Medicago sativa* and two species of *Melilotus*. This occurred within 6 days after inoculation, usually on leaves which were lesioned very severely. In nature, pycnidia of *A. imperfecta* rarely develop on leaves closely following infection.

TABLE I

Isolations of *Ascochyta imperfecta* from Canadian alfalfa seed (1958 crop)

| Source of seed | Number of samples | Number of samples infected | % of seeds yielding colonies |
|---|---|---|---|
| Ontario | 4 | 2 | 10 |
| Manitoba | 11 | 9 | 61 |
| Saskatchewan | 24 | 20 | 38 |
| Alberta | 17 | 12 | 23 |
| British Columbia | 8 | 7 | 20 |

PLATE I

FIG. 1. Scale of lesioning used in excised-leaf tests with *Ascochyta imperfecta*. Top center 0; clockwise 1, 2, 3, 4, 5.

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*Pathogenicity of the Isolates*

As expected, there were distinct differences in the reaction of the 10 hosts to *A. imperfecta*. *Medicago sativa* was most susceptible, and within this species the variety Grimm was more severely attacked than the two hybrids S-57-76 and S-57-139. *M. falcata*, under natural conditions, is quite resistant to *A. imperfecta*; in the excised-leaf tests it was susceptible to most of the isolates, but less so than *M. sativa*. *Melilotus alba*, *M. officinalis*, and *Trifolium pratense* ordinarily are not hosts to *A. imperfecta*, but in these tests they were moderately susceptible, *T. pratense* being least so. *Pisum sativum* was similar to *M. falcata* in susceptibility and *Vicia faba* similar to *T. pratense*. *Lotus corniculatus* was highly resistant to all isolates tested.

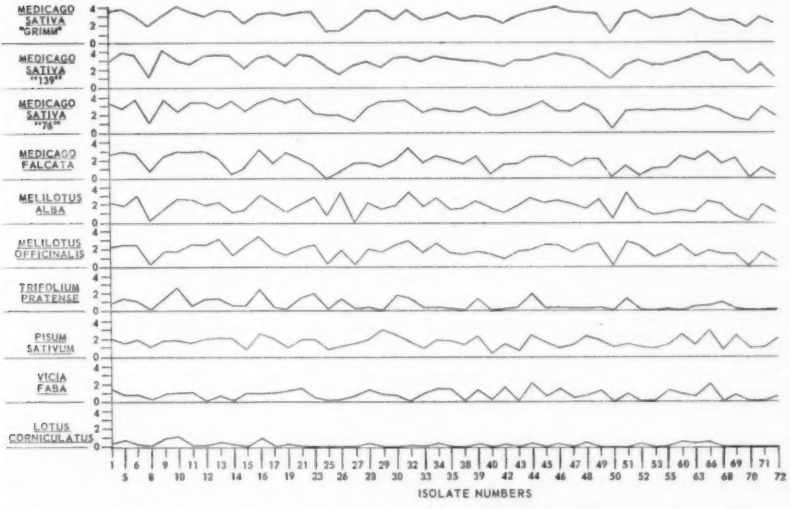


FIG. 2. Disease ratings from leguminous hosts inoculated with *Ascochyta imperfecta*.

The 50 isolates varied in pathogenicity from very weak to very strong, most of them being moderately pathogenic (Fig. 2). Usually where an isolate was weak on one host, it was weak on others, but there were exceptions. For example, isolate 32 was one of the weakest on *V. faba* but one of the strongest on four other hosts. Isolate 52 was very weak on *M. falcata*, *T. pratense*, and *V. faba*, but of about average pathogenicity on the other hosts. Other examples are isolates 5 and 27, which were much weaker on *M. sativa* "76" than on "139" and "Grimm". Both species of *Melilotus* reacted similarly to all 50 isolates.

The disease ratings represented in Fig. 2 were analyzed to determine the significance of the variations in pathogenicity of the 50 isolates. When all hosts were included (Table II), the *F* tests indicated that there were significant differences in susceptibility between the various hosts. The interaction,

TABLE II

Analysis of variance of disease ratings with *A. imperfecta* on 10 hosts

| Source of variation | Degrees of freedon | Mean square | *F* |
|---|---|---|---|
| Hosts | 9 | 48.2660 | 156.00** |
| Hosts (*a*) *Medicago, Melilotus*, others | 6 | 71.7503 | 185.49** |
| Hosts (*b*) within *M. sativa* | 2 | 1.9460 | 11.62** |
| Hosts (*c*) within *Melilotus* | 1 | 0.0001 | — |
| Isolates | 49 | 2.7815 | 8.99** |
| Isolates × hosts | 441 | 0.3094 | |
| Isolates × hosts (*a*) | 294 | 0.3868 | 2.50** |
| Isolates × hosts (*b* and *c*) | 147 | 0.1545 | |
| Isolates × hosts (*b*) | 98 | 0.1683 | |
| Isolates × hosts (*c*) | 49 | 0.1268 | |

**Significant at the 1% level.

TABLE III

Analysis of variance of disease ratings with *A. imperfecta* on *Medicago sativa* and *M. falcata*

| Source of variation | Degrees of freedom | Mean square | *F* |
|---|---|---|---|
| Total | 799 | 16.0788 | |
| Replicates | 3 | 8.3333 | |
| Isolates | 49 | 116.6734 | 7.68** |
| Groups | | | |
| *M. falcata* vs. *M. sativa* | 1 | 2970.3750 | 123.95** |
| Between strains of *M. sativa* | 2 | 111.9650 | 10.37** |
| Isolates × groups | 147 | 15.1884 | 5.40** |
| Isolates × strains of *M. sativa* | 98 | 10.8000 | 3.84** |
| Isolates × species | 49 | 23.9651 | 8.53** |
| Error | 597 | 2.8107 | |

**Significant at the 1% point.

isolates × hosts (*a*), tested against the mean square for isolates × hosts (*b* and *c*) was considered to be the best test of significance for interaction, isolates × hosts. The analysis showed the *F* value for this test (2.50) to be highly significant ($P$,.01). This test clearly demonstrated specificity among the isolates. In this interaction, all of the strains of *Medicago* were considered as a single group so that no light was thrown on the interaction of the three strains with the isolates. In a second analysis (Table III), comparisons were made between *M. falcata* and *M. sativa* and between the three strains of *M. sativa*. The *F* values in these tests were highly significant, indicating over-all differences between the two species, and also differences between strains of *M. sativa*. The tests further indicated significant differences between species of *Medicago* in their reaction to different isolates, and also differences between strains of *M. sativa* in this respect. Such specificity is interpreted to mean that parasitic strains of *A. imperfecta* existed among the 50 isolates.

## Discussion

The present demonstration of the existence of parasitic strains among isolates of *Ascochyta imperfecta* may explain partially the variation in sus-

ceptibility to that fungus of selected strains of alfalfa at various locations (6). Similarly, the differences obtained in field tests under natural conditions, and in inoculation tests with excised leaves, as reported by Mead and Downey (5), are more understandable when specificity among isolates is known to exist. Such variation makes the use of numerous isolates obligatory in inoculation of breeding stock of alfalfa with *A. imperfecta*.

The relative susceptibility of the 10 leguminous hosts in this study was similar to that reported in field and greenhouse inoculation trials by other workers (2, 4, 7, 9). The most susceptible hosts were species of *Medicago*, *Melilotus*, and *Trifolium*. Exceptions to the present findings were those of Cormack (1) and Toovey *et al.* (7), who found that plants of *Pisum sativum* and *Vicia faba* were not susceptible to *A. imperfecta*. In the present study, excised leaves of *P. sativum* were susceptible to all of 50 isolates, and leaves of *V. faba* to 39 of them. *Lotus corniculatus* was highly resistant to *A. imperfecta* in this study.

The widespread occurrence of *A. imperfecta* on alfalfa seed in Canada is illustrated by its presence on 50 to 70% of the samples from each of the five provinces (Table I). The effect of weather conditions on the severity of seed infestation is shown in the data from Manitoba seed. This seed was exposed to wet fall weather and overwintering in the field.

## Acknowledgment

The writers are indebted to Dr. H. B. Cannon, Statistical Research Service, Research Branch, Canada Department of Agriculture, for statistical analysis and interpretation of the data from pathogenicity tests.

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# FACTORS AFFECTING BRANCH INFECTION IN ASPEN[1]

D. E. ETHERIDGE[2]

## Abstract

Comparative studies have shown that branch infections are almost twice as frequent in aspen (*Populus tremuloides* Michx.) in mesic sites as in trees of similar ages in dry sites. This has been attributed to: (1) the proneness to infection of young dead branches, and (2) the greater mortality of young branches in mesic sites. No difference was found between the two sites with respect to the pattern of air temperature and relative humidity. The moisture content of dead branches was influenced to a minor extent by site, but only after considerable rain had fallen did branch moisture reach a level favorable for infection by fungi. Although several species of imperfect fungi and bacteria infected aspen branches from 4 years following mortality, wood-destroying fungi, namely *Corticium polygonium* Pers. and *Polyporus adustus* (Willd.) Fr., did not appear until 7 and 8 years after the death of branches. After 19 years, decay of *Fomes igniarius* (L. ex Fr.) Kickx. occurred in branches only as lateral extensions of heartwood infection. The feasibility of artificial pruning to reduce the risk of heart rot infection in young aspen stands is discussed.

## Introduction

It has been suggested that artificial pruning of pole-sized aspen might reduce the high incidence of heart rot infection that seriously limits utilization of this species in some regions (7). This assumes that branch stubs furnish the avenues of entrance for the majority of trunk-rot infections in aspen, and that removing potential infection courts at an age before they become effective might control losses resulting from heart rot. As a result of recent studies in Alberta, detailed information is available on the occurrence and relative importance of the fungi associated with decay in aspen (8), but the exact mode of entrance and the conditions affecting the establishment of fungi in the heartwood are still largely unknown.

Investigations were undertaken in Alberta to study the course of infection in living and dead branches of aspen. The principal objective was to obtain information regarding the time and conditions when dead branches might serve as entrance points for heartwood fungi, but information was also sought concerning such factors as site, tree vigor, and local climate in relation to the infection process.

## Location and Description of Study Area

The investigation was undertaken in the summer of 1958 near Whitecourt, Alberta, about 100 miles northwest of Edmonton. The study area is part of the Lower Foothills Section of the Boreal Forest Region. Extensive studies of the decays and resulting losses in aspen and balsam poplar were made in the same general region (7, 8) during 1957 and 1958. The characteristic tree

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species of the area are lodgepole pine, *Pinus contorta* Dougl. var. *latifolia* Engelm.; trembling aspen, *Populus tremuloides* Michx.; and balsam poplar, *P. balsamifera* L. Most of the stands of the area have originated after fires.

Plots were established in a pure aspen and a mixed aspen – balsam poplar stand that were considered to be representative of dry and mesic sites for aspen of this region. The pure stand was located on a well-drained ridge and the mixed stand was on the lower slope of the same ridge, at a distance of about one-quarter mile. Both stands were the same age (34 years) and of equal density (700 stems per acre[3]), but differed in their rate of growth and ground vegetation. The pure stand (95% aspen) was characterized by an average height and diameter of 45 ft and 5.0 in. (d.b.h.), respectively, and by the presence of *Shepherdia canadensis* (L.) Nutt., *Arctostaphylos uva-ursi* (L.) Spreng., and *Lathyrus ochroleucus* Hook. The mixed stand (44% aspen) was characterized by superior height and diameter growth, namely 51 ft and 5.9 in. (d.b.h.), respectively, and by a dense ground cover consisting largely of *Amelanchier alnifolia* Nutt., *Salix* sp., and *Aralia nudicaulis* L.

## Methods

Three dominant aspen trees were selected in each of the two stands for detailed examination and for the purpose of making continuous observations on environmental factors during the growing season. About twenty-five intact and broken branches were tagged and numbered between the butt and the living crown of each sample tree. These provided a variety of branches of different sizes, ages, and stages of deterioration which could be assessed in terms of local environment and, later, in terms of the associated mycoflora.

Atmospheric temperature and moisture data were taken at 5-ft intervals on the stems of sample trees up to the height of living crowns for a 5-week period in July and August. Air temperature was measured at each point on the stems, using dial-type thermometers which were attached with the sensitive end resting on the surface of the bark. These measurements were taken for N.E. and S.W. aspects. The thermometers were calibrated to give comparable readings at 0 and 100° C. Relative humidity of the air was measured close to the stem at each 5-ft interval, using a sling-type psychrometer. Weekly records of moisture changes in selected branches were obtained by employing a non-destructive method of determining the moisture content of wood. This method (2) was found to be sensitive to the range of moisture values encountered in the present study.

Aluminum extension ladders were used to obtain direct measurements of environmental factors and for periodic sampling of trees. The unit consisted of two 24-ft ladders held rigidly together with braces and provided with guy ropes which were used to lash it to the tree. When firmly braced the whole unit could be moved from tree to tree, and manoeuvred into position in about 10 minutes by three men.

The occurrence of branch-inhabiting organisms on the six sample trees was determined at the conclusion of the season's observations, on the basis of isolations made from branches at their point of juncture with the stem.

[3]Stems having a diameter at breast height (d.b.h.) greater than 2.5 in.

Sections taken from the base of each branch were split through the center with a sterile scalpel, and three small pieces of wood and pith were removed from successive points, about one-quarter inch apart, and placed on 2.5% malt agar slants. The same technique of making isolations was followed for supplementary sampling of branches and dead terminals elsewhere in the stands.

The ages and the times of death of branches were determined by counting the number of annual rings at the base of each branch, and comparing these ages with those of adjacent stem sections. The differences between these values were assumed to equal the number of years that had elapsed since the branches had died.

The data were entered on punched cards for ease of compilation.

## Results

*Effect of Site on Occlusion of Branch Bases*

Comparative studies of aspen on dry and mesic sites in Alberta have shown that trees on the moister site grew much faster. This suggests that in the more vigorous trees there occurred a more rapid growing-over of branch stubs, with a corresponding reduction in the length of time that they were exposed to infection. Information on the time required for trees to cover branch bases of various lengths was obtained by dissecting a total of 73 occluded branches in mature aspen growing in dry and mesic sites. The results are presented in Fig. 1, which shows that branch occlusion proceeds at a much slower rate for

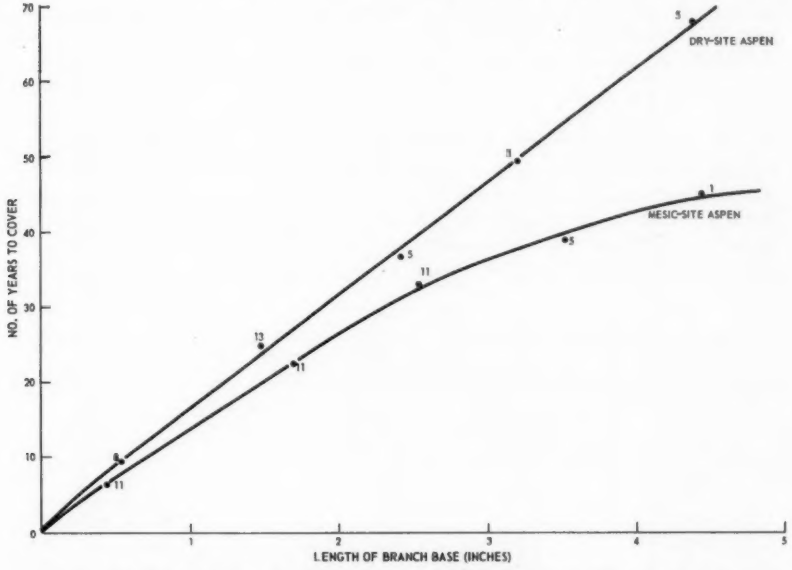


FIG. 1. Relative persistence of branch bases (stubs) on aspen in dry and mesic sites.

TABLE I

Mean temperature (°F) and relative humidities for a 5-week period in July and August at various heights and positions on two aspen trees in a dry and a mesic site at Whitecourt, Alberta

| | Position of recording instruments (distance from ground, feet) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 5 | | 10 | | 15 | | 20 | | 25 | | Base of living crown | | Mean | | Total mean | Temp. |
| Item | NE. | SW. | NE. | SW. | NE. | SW. | NE. | SW. | NE. | SW. | NE. | SW. | NE. | SW. | NE. | SW. | NE. and SW. | Min. Max. |
| **Dry site** | | | | | | | | | | | | | | | | | | |
| Temp. | | | | | | | | | | | | | | | | | | |
| (a.m.) | 66.2 | 63.2 | 64.4 | 62.5 | 63.6 | 64.2 | 66.3 | 62.3 | 64.1 | 62.3 | 65.2 | 61.9 | 67.8 | 62.7 | 65.4 | 62.7 | 64.1 | |
| (p.m.) | 69.8 | 75.5 | 73.3 | 75.6 | 72.4 | 76.0 | 73.5 | 74.6 | 73.0 | 75.6 | 72.2 | 75.7 | 76.9 | 73.8 | 73.0 | 75.3 | 74.1 | 45.3 81.2 |
| Mean | 68.0 | 69.1 | 68.7 | 69.0 | 67.9 | 69.9 | 69.9 | 68.4 | 68.5 | 68.8 | 68.7 | 68.7 | 72.3 | 68.2 | 69.1 | 68.9 | 69.0 | 69.3 |
| Relative humidity | | | | | | | | | | | | | | | | | | |
| (a.m.) | 65.4 | | 60.7 | | 60.2 | | 59.7 | | 59.4 | | 59.3 | | | | | | 60.8 | |
| (p.m.) | 49.2 | | 44.5 | | 43.0 | | 42.9 | | 42.4 | | 42.4 | | | | | | 44.1 | |
| Mean | 57.3 | | 52.6 | | 51.8 | | 51.4 | | 51.0 | | 51.0 | | | | | | 52.6 | |
| **Mesic site** | | | | | | | | | | | | | | | | | | |
| Temp. | | | | | | | | | | | | | | | | | | |
| (a.m.) | 66.5 | 61.2 | 65.4 | 63.2 | 63.9 | 60.4 | 65.3 | 61.9 | 62.6 | 63.0 | 61.9 | 62.8 | 62.8 | 61.9 | 63.9 | 62.0 | 63.0 | |
| (p.m.) | 69.5 | 68.3 | 74.2 | 75.6 | 72.8 | 73.6 | 73.3 | 76.0 | 73.0 | 77.1 | 71.8 | 75.6 | 73.3 | 76.8 | 72.5 | 74.6 | 73.6 | 45.5 83.4 |
| Mean | 68.0 | 64.7 | 69.8 | 69.3 | 68.1 | 66.7 | 68.5 | 68.7 | 67.6 | 69.8 | 66.6 | 68.9 | 67.8 | 69.0 | 68.1 | 68.1 | 68.1 | 64.4 |
| Relative humidity | | | | | | | | | | | | | | | | | | |
| (a.m.) | 62.3 | | 59.5 | | 58.1 | | 57.8 | | 58.1 | | 56.9 | | | | | | 59.2 | |
| (p.m.) | 51.1 | | 45.7 | | 43.2 | | 42.4 | | 42.0 | | 40.7 | | | | | | 44.3 | |
| Mean | 56.7 | | 52.5 | | 50.8 | | 50.1 | | 50.0 | | 49.9 | | | | | | 51.8 | |
| **Temperature** | | | | | | | | | | | | | | | | | | |
| Mean | 68.0 | 66.9 | 69.2 | 69.1 | 68.0 | 68.3 | 69.2 | 68.5 | 68.1 | 69.3 | 67.6 | 68.8 | 70.1 | 68.6 | 68.6 | 68.5 | 68.6 | |
| **Relative humidity** | | | | | | | | | | | | | | | | | | |
| Mean | 57.0 | | 52.5 | | 51.3 | | 50.8 | | 50.5 | | 50.5 | | | | | | 52.2 | |

NOTE: Vapor pressure deficit: dry site, 8.58 mm; mesic site, 8.47 mm.

dry-site aspen than for mesic-site aspen. For example, while branch bases of 1-in. length are covered in mesic sites in 13½ years, a further 3 years are needed to accomplish this in dry sites. Furthermore, the differential between the two sites in the time required for branch stubs to heal increases with increasing length of branch stubs, and in the present study reached a maximum of about 20 years for branch bases that measured 4 in. in length.

*Temperature and Relative Humidity in Relation to Site*

To determine whether differences occurred in the local climate, air temperature and relative humidity were measured on one tree in each site. The measurements were taken twice daily in the manner described earlier. In addition, maximum and minimum temperatures were recorded daily at heights of 0, 15, and 25 ft on the two trees. Table I summarizes the mean values of air temperature and relative humidity for the 5-week period, and shows a fairly uniform pattern for both climatic factors at comparable recording positions in the two areas. The positional and temporal fluctuations of temperature that occurred were for the most part attributed to the relative position of the sun and the recording instruments, maximum values being obtained in both areas during the afternoon on the southwest side of the trees. Relative humidities were highest in the morning and lowest in the afternoon, with maximum values obtaining in both sites below the 5-ft level of the trees. Average temperatures over the 5 weeks were slightly higher in the dry site (69.0° F) than in the mesic site (68.1° F). However, comparisons of the mean maximum (day) and minimum (night) temperatures for each site reversed this trend, the higher temperatures occurred in the mesic site. Only small differences occurred between the average relative humidities of the two sites. Slightly higher values were recorded in the morning in the dry site and in the afternoon in the mesic site.

*Branch Moisture Content in Relation to Site*

It is generally known that seasonal and spatial fluctuations occur in the moisture content of trees, and in some instances the pattern of distribution of moisture in the stems can be related to site. On the other hand, the moisture relations of dead branch material are less well known, and there is little pertinent information on how these are influenced by local environmental conditions. To ascertain if differences in branch moisture existed between the

TABLE II

Moisture content of selected dead branches of aspen in a dry and a mesic site near Whitecourt, Alberta

| Site | Designation of sample tree | No. of branches sampled | Average no. of determinations per branch | Average moisture content (% o.d.w.) |
|---|---|---|---|---|
| Dry | 3501 | 18 | 3.4 | 16.5 |
| | 3502 | 15 | 3.9 | 16.1 |
| Total and averages | | 33 | 3.6 | 16.3 |
| Mesic | 3504 | 11 | 3.5 | 20.0 |
| | 3505 | 15 | 2.8 | 19.2 |
| Total and averages | | 26 | 3.1 | 19.6 |

two areas, weekly measurements were made of the moisture content of selected dead branches for a 4-week period in July and August. A total of 33 branches in the dry site and 26 branches in the mesic site provided data for the calculation of average moisture contents for this period (Table II).

Dead branches in both areas were relatively dry during the period under investigation; moisture values were substantially lower than the fiber saturation level (about 27% o.d.w.). An average moisture content of 19.6% occurred in the branches of the two mesic-site trees as compared with a value of 16.3% for the two dry-site trees. This represents a difference of 3.3% between the moisture means of the two areas, which is significant at the 5% level of probability.

*The Effect of Periodic Rainfall on the Moisture Content of Dead Branches*

Early in the investigation it was apparent that rain had an immediate and marked effect on the moisture content of dead branches. Consequently, an attempt was made to study the nature of this relationship, by comparing the amount and frequency of rainfall with changes in the moisture content of a typical dead branch. Rainfall was measured with a standard meteorological-type rain gauge which was placed in an open space about 50 ft from the experimental branch, and observations were made daily at 8.30 a.m., or at the cessation of rain. The reason for installing the rain gauge in an open space was to simulate standard meteorological methods of measuring rainfall, so that if a correlation was obtained, the influence of climatological moisture on the infection process might be assessed later from published rainfall records. For the purpose of recording daily changes in branch moisture a freshly detached dead branch was installed about 5 ft from the ground between two trees in the dry site. Measurements were made by the same method used for determining the moisture content of naturally occurring dead branches. Fourteen moisture probes were inserted in holes bored partially through the basal portion of the selected branch. Two were removed each day at 8.30 a.m. for the purpose of recording the moisture changes in the sample. The experiment was continued for the period July 15–August 19.

The results showing the relationship between rainfall on the forest cover and branch moisture content are presented in Fig. 2. While there is evidence of a direct relationship between the two variables, the amount of increase in branch moisture was not exactly proportional to the actual amount of rain that was collected. For example, the .06 in. of rain measured on July 22 corresponded to an increase in branch moisture from 17.5% to 29.5%, a net gain of 12.0%. About the same amount of rain on August 9 (.07 in.) produced a change in the moisture level of the branch from 16.5% to 23.5%, representing a net gain of only 7.0%. Following a still greater amount of rain on August 14 (.09 in.), the amount of moisture in the branch increased by only about the same percentage, from 14.0% to 21.5%. These data suggest the existence of other variables, among which fluctuations of relative humidity, temperature, and duration and direction of the rain might have played a part. Attempts to discover a relationship between either daily means of temperature or relative humidity and moisture trends were unsuccessful. Since precipitation occurred mainly at night, there were no data available for assessing possible

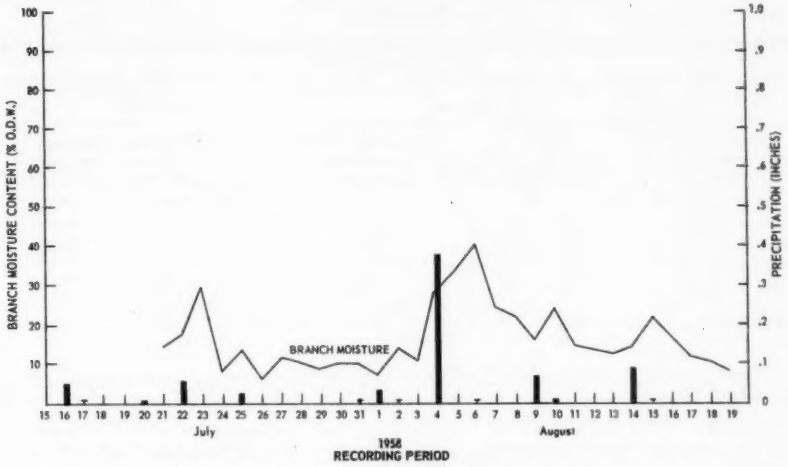


FIG. 2. Amount of rainfall (vertical bars) in relation to branch moisture (curve).

influences of variable rainfall periods. It can be assumed, however, that the intensity and duration of rain would have an important effect on the amount of moisture imbibed by dead branch material.

Maximum moisture content values were not obtained for the experimental branch until from 1 to 2 days after each rain. This lag is thought to represent the length of time required for moisture to penetrate to the center of the branch, where moisture measurements were made. When rainfall was light (less than .01 in.) moisture penetration under the forest cover was negligible, and measurable changes in moisture levels did not occur in the branches.

An interesting result of the experiment was that there were only 4 days during the 29-day period in which the branch moisture level exceeded fiber saturation level (27% o.d.w.). Fiber saturation level compares closely to the minimum wood moisture content (about 25%) that is suitable for the development of several aspen branch-inhabiting fungi (4). It appears, therefore, that conditions permitting branch infections may be relatively infrequent in the study area.

The mean moisture value (15.9%) for the detached branch over the observational period compared favorably with that for naturally occurring dead branches (16.3%) in the same area (see Table II).

*The Incidence of Branch-infecting Organisms in Relation to Site*

The number of years since the death of each branch was determined before the incidence of branch infection in the two sites could be assessed. This became necessary since the branches had been dead for varying lengths of time, and therefore had been exposed to infection for varying periods. Branch samples for the two sites were then compared, with respect to the number of different organisms they yielded in culture at different ages after their death. The results of these comparisons are given in Table III.

TABLE III

Relation between the number of infections and years since death of branches in aspen in a dry and a mesic site

| No. of years since death of branches | Dry site | | | Mesic site | | |
|---|---|---|---|---|---|---|
| | No. of branches | No. of infections | Average no. infections per branch | No. of branches | No. of infections | Average no. infections per branch |
| 13–16 | 30 | 8 | 0.26 | 15 | 6 | 0.40 |
| 17–20 | 26 | 13 | 0.50 | 15 | 10 | 0.67 |
| 21–24 | 35 | 20 | 0.57 | 15 | 18 | 1.20 |
| 25–28 | 11 | 9 | 0.82 | 11 | 16 | 1.45 |
| 29–32 | 2 | 2 | 1.00 | — | — | — |
| Totals and averages | 104 | 52 | 0.50 | 56 | 50 | 0.89 |

Examples of branches that had died during the 12 years immediately prior to 1958 were not found in the mesic site, and are therefore excluded from Table III. Over the period for which there were comparable data there was a consistent and marked increase in the number of infections in both sites for each successive 4-year period. More infections occurred in mesic-site branches than in dry-site branches at comparable ages. The average number of branch infections in the mesic site was about twice that for the dry site, 0.89 and 0.50 infections per branch respectively.

## Conditions Affecting Infection of Branches by Fungi

*The Effect on Infection of Time Since the Death of Branches*

Other factors being favorable, the infection of dead branches may be considered as a continuous process until the residual branch base is completely embedded in the living tissues of the stem. Thus, the element of time must be a basic consideration when assessing the relative importance of different branch characteristics on the infection process. The relation between the incidence of branch infection and the length of time that branches had been dead are given in Table IV.

There is a general tendency for the percentage of infected branches to increase with the number of years since mortality, but not at a uniform rate. In the present sample, a sharp increase occurred in the number of newly infected branches in the first 8 years after death. This was followed by a period of relatively few infections in still older branches. This in turn was followed by greatly increased infections in the oldest branches sampled. While it is possible that differences in rainfall over the period might have accounted for some of the observed inconsistencies, they were generally attributed to irregularities in the sample, and probably would have been less significant had the branches been completely comparable as to size and general condition. This is evident from the analyses that follow.

*Breakage of Branches*

Earlier studies of the infection process in aspen demonstrated that broken branches (stubs, Fig. 6) were twice as likely to contain saprophytic organisms as intact branches, but the reason for this was not determined (3). The present

TABLE IV

Incidence of infection in relation to the number of years since the death of branches

| Years since death of branches | No. of branches | No. of infected branches | % of branches infected |
|---|---|---|---|
| 0–4 (1954–1958) | 17 | 3 | 17.7 |
| 5–8 (1950–1954) | 52 | 23 | 44.2 |
| 9–12 (1946–1950) | 49 | 22 | 45.0 |
| 13–16 (1942–1946) | 49 | 16 | 32.7 |
| 17–20 (1938–1942) | 48 | 24 | 50.0 |
| 21–24 (1934–1938) | 51 | 30 | 58.8 |
| 25–28 (1930–1934) | 21 | 17 | 81.0 |
| 29–32 (1926–1930) | 3 | 3 | 100.0 |
| Totals and average | 290 | 138 | 47.6 |

studies afforded an opportunity to confirm these findings, and to investigate additional factors that might explain them. The data were arranged in 4-year classes according to the year of mortality. The numbers of infected branches were expressed as percentages of the total number of broken and intact branches that occurred in each mortality class (Table V).

Together with a general trend of increased infections with the passage of time, an appreciably higher incidence of infected branches occurred in the "broken" category (61.9%) than in the "intact" category (41.1%), when

TABLE V

Frequency of infection in dead branches in relation to their condition: broken or intact

| Years since death of branches | Intact branches | | | Broken branches | | |
|---|---|---|---|---|---|---|
| | No. | No. inf. | % inf. | No. | No. inf. | % inf. |
| 0– 4 | 8 | 0 | 0 | 3 | 1 | 33.3 |
| 5– 8 | 40 | 16 | 40.0 | 11 | 6 | 54.5 |
| 9–12 | 45 | 17 | 37.8 | 4 | 3 | 75.0 |
| 13–16 | 37 | 11 | 29.7 | 12 | 5 | 41.5 |
| 17–20 | 34 | 15 | 44.1 | 14 | 9 | 64.2 |
| 21–24 | 35 | 18 | 51.4 | 16 | 12 | 75.0 |
| 25–28 | 10 | 9 | 90.0 | 11 | 8 | 72.8 |
| Totals and averages | 209 | 86 | 41.1 | 71 | 44 | 61.9 |

branches of the same mortality classes were compared. Of the branches that had died during the 4 years prior to 1958 more than 30% classed as broken were infected. None of the intact branches of the same class contained saprophytic organisms. The same trend was evident in the older mortality classes, indicating that infection is partly influenced by a factor or factors associated with breakage.

*Branch Size*

It was noted that the larger dead branches had generally remained intact longer than smaller ones, suggesting that either the size of a branch or its age could influence the incidence of infection in branch stubs. This possibility was tested by considering branch diameter and branch condition, whether broken or intact, in relation to infection (Table VI).

TABLE VI

Relation between branch diameter,* condition of branches (broken or intact), and infection

| Branch size (diameter in in.) | Branch condition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Broken | | | | Intact | | | |
| | No. | % of total | No. inf. | % inf. | No. | % of total | No. inf. | % inf. |
| Less than 0.5 | 50 | 39.0 | 33 | 66.0 | 78 | 61.0 | 46 | 59.0 |
| 0.5–0.8 | 14 | 11.3 | 5 | 36.0 | 110 | 88.7 | 37 | 33.6 |
| More than 0.8 | 2 | 6.5 | 2 | 100.0 | 29 | 93.5 | 9 | 31.0 |
| Totals | 66 | | 40 | | 217 | | 92 | |

*Diameter outside bark in inches measured at the proximal end of the branch.

Although a disproportionate number of intact and small branches occurred in the sample, a relationship was obtained between branch size and breakage, sufficient to indicate that large branches are more resistant to breakage than small branches. For example, the proportion of broken branches in the total sample decreased from 39% for the small class (less than 0.5 in diameter) to 6.5% for the large class (more than 0.8 in diameter).

More than half of the branches having a diameter of less than 0.5 in. were infected, but only about one-third of those of a larger size class (0.5 to 0.8 in.) were infected. Thus, intact or broken branches that had died before attaining a diameter of 0.5 in. appeared to have been particularly prone to infection. The fact that three-quarters of all of the broken branches examined were less than 0.5 in. in diameter and only one-third of the intact branches were of this size suggests that branch size, rather than breakage of the branches, is more important from the standpoint of infection.

*Age and Growth Rate of Branches*

Since branch size is a function of both age and growth rate, either of these factors could be related to the increased infections observed for small, dead branches. The possible effect of branch age at the time of death was examined by comparing the incidence of infection in branches that had up to five growth rings with branches having more than five growth rings (Table VII). A

TABLE VII

The susceptibility to infection of branches in relation to their size and time of death

| Years since death of branches | No. of annual growth rings in dead branches | | | | | |
|---|---|---|---|---|---|---|
| | 5 or less | | | 6 or more | | |
| | Total no. of branches | No. inf. | % inf. | Total no. of branches | No. inf. | % inf. |
| 0–10 (1948–1958) | 45 | 27 | 60.2 | 79 | 26 | 32.8 |
| 11–20 (1938–1947) | 17 | 10 | 58.8 | 106 | 45 | 42.4 |
| 21–30 (1928–1937) | 22 | 19 | 86.4 | 47 | 27 | 57.4 |
| Totals and averages | 84 | 56 | 66.6 | 232 | 98 | 42.2 |

TABLE VIII

The incidence of infection in branches in relation to their rate of growth

| Average width of growth rings (in.) | Total no. of branches | No. infected branches | % of branches infected |
|---|---|---|---|
| Less than .021 | 44 | 24 | 54.5 |
| .021–.030 | 84 | 49 | 58.3 |
| .031–.040 | 87 | 34 | 39.2 |
| .041–.050 | 71 | 34 | 47.9 |
| .051–.060 | 9 | 5 | 55.5 |
| .061–.070 | 6 | 1 | 16.7 |
| .071–.080 | 6 | 4 | 66.5 |
| More than .080 | 6 | 2 | 33.3 |
| Totals and average | 313 | 153 | 48.8 |

consistently higher rate of infection had occurred in the younger branches, regardless of the number of years that had elapsed since their death. The effect of growth rate was determined by comparing the relative susceptibilities of branches having varying widths of annual rings (Table VIII). No relation could be found between branch infection and the width of growth rings. On the contrary, branches were uniformly infected regardless of their growth rate.

*Branch Position*

A comparison of the incidence of infection in branches occupying the south and west sides of stems, with those on the north and east sides, revealed a uniform distribution of infected branches for all aspects. No attempt was made to determine whether the frequency of infection varied with the distance of branches above the ground, since this involved branch age and mortality, which are factors directly related to infection. Except near ground level environmental conditions were fairly uniform along the stem and would be unlikely to play an important part in the infection process.

## Fungi Isolated from Aspen

A total of 1056 isolations were attempted in the course of the investigation, 867 from branches, 108 from old leader shoots, and 81 from stem lesions and decayed wood. Three hundred and sixty-six of these yielded cultures of microorganisms, 22 of which were rejected because they represented aerial contaminations. The frequencies of isolation of successful cultures are shown in Table IX. The cultural characteristics of the identified fungi have been previously described in the literature. A large number of the cultures were obtained either singly or relatively rarely; these have been grouped together and designated as miscellaneous isolates. No attempt was made to identify the bacteria. The more frequently occurring fungi for which specific identification has not been possible include several species of *Cytospora*, a species of *Libertella*, and a species of *Phoma*. The cultural characteristics of these species are discussed hereunder.

TABLE IX

Frequency of isolation of fungi and bacteria from branches, terminal shoots, and stems* of aspen at Whitecourt, Alberta

| Fungus | No. of isolations | | | |
|---|---|---|---|---|
| | Branches | Terminal shoots | Stems | Totals |
| *Corticium polygonium* Pers. | 53 | 0 | 3 | 56 |
| *Coryne* sp.† | 0 | 0 | 2 | 2 |
| *Cytospora chrysosperma* (Pers.) Fr. | 29 | 6 | 7 | 42 |
| *Cytospora* spp. | 53 | 16 | 0 | 69 |
| *Fomes igniarius* (L. ex Fr.) Kickx. | 3 | 0 | 1 | 4 |
| *Libertella* sp. | 10 | 1 | 0 | 11 |
| *Phoma* sp. | 39 | 2 | 1 | 42 |
| *Polyporus adustus* Willd. ex Fr. | 3 | 0 | 0 | 3 |
| *Polyporus zonatus* Fr. | 0 | 2 | 0 | 2 |
| *Stereum purpureum* (Pers. ex Fr.) Fr. | 0 | 0 | 1 | 1 |
| Bacteria | 16 | 3 | 4 | 23 |
| Miscellaneous | 73 | 15 | 1 | 89 |
| Totals | 279 | 45 | 20 | 344 |

*Stem isolations include bark lesions and decayed wood.
†Tentatively identified as *Coryne microspora* Ell. & Ev. (*Ombrophila microspora*) (Ell. & Ev.) Sacc. & Syd. by Mrs. Doreen Wilson, formerly Associate Mycologist, Plant Research Institute, Ottawa.

*Cytospora* spp.—The several different culture types included under this designation probably represent a complex of two or three distinct species of *Cytospora*. Colonies on malt agar show considerable variation in growth rate, some isolates covering the entire plate in 2 weeks, others in 3 weeks, while a few isolates were moderately slow-growing, attaining a colony diameter of only 2 to 3 cm in 3 weeks. In gross appearance, the cultures are similar; colonies on malt agar are white to grey at first, becoming black as they mature. Fruiting structures have not been obtained from all the isolates. The pycnidiospores are hyaline, cylindrical to elliptical, generally larger than those of *Cytospora chrysosperma*, measuring 1.5–3.0 $\mu$ × 4.5–6.7 $\mu$. The cultures also differ from *C. chrysosperma* in their absence of yellow spore tendrils and in the characteristic black appearance of colonies on malt agar.

Reference collection: DAOM 60790, 60791 at Plant Research Institute, Ottawa. Isolates with similar cultural characteristics have previously been designated "BC" (Etheridge and Laut (3)).

*Libertella* sp.—Cultures of this fungus have been obtained from decay in *Populus* spp. in Alberta and elsewhere in Canada, but have not been identified as to species (Nobles, letter, 1957). The gross cultural characteristics of this fungus are described by Fritz (5).

*Phoma* sp.—Colonies on malt agar grow slowly, attain a diameter of 3.3 cm in 3 weeks at room temperature (24° C), have a smooth, white, opaque margin that is mostly submerged and has lightly scattered tufts of aerial mycelium in some cultures. The colonies become green at the center with the formation of scattered pycnidia. Hyphae of the advancing zone are hyaline, 2.0–3.6 $\mu$ in diameter. The surface mycelium is hyaline, 2.0–4.5 $\mu \times$ 9.0–33.0 $\mu$, becoming green about short broad-celled pycnidial initials, 3.0–4.5 $\mu \times$ 3.0–9.0 $\mu$. The spores are hyaline, cylindrical to allantoid, commonly ligutulate, 1.8–3.6 $\mu \times$ 4.5–6.5 $\mu$, and are produced on hyphae or in pycnidia. The pycnidia are black, very small, measuring 90 $\times$ 140 $\mu$, neck 50–60 $\mu$ in diameter (on wood), and are partly submerged in plate cultures. The spores are discharged in a milky, off-white droplet.

Reference collection: DAOM 57112 at Plant Research Institute, Ottawa.

The relative frequency of isolation of the organisms from branches of different ages provided data on the probable course of succession. The successional pattern is illustrated in Table X, which indicates five distinct stages of colonization in aspen branches. The distribution of the selected species in branches, dead terminals, and stems are shown in Table IX.

*Stage 1.*—The earliest stage of colonization was characterized by bacterial infections, which occurred in two of the five living branches sampled. Isolation of bacteria from branches which had been dead for periods up to 22 years indicated that there had been no general replacement of this group by later groups of colonizing fungi. Bacteria showed no preference for specific parts of the sample trees, and were found in association with several different species of later-colonizing fungi.

*Stage 2.*—Colonization by fungi did not occur until 4 years following the death of branches. *Cytospora* spp., which completely dominated the infected branches during this stage, were also the most frequently occurring organisms of later stages. This group of fungi was confined to dead branches and old terminals (Figs. 3, 4, 5).

*Stage 3.*—*Cytospora chrysosperma*, *Phoma* sp., *Libertella* sp., and several species of the miscellaneous group of fungi entered the succession 6 years following branch mortality. Except for *Libertella*, which was infrequently isolated from dead branches, fungi of this group occurred fairly consistently among later infections. *C. chrysosperma* showed a greater ability to colonize stem tissues than the other fungi. This is in general agreement with its frequent association with decay organisms in mature aspen.

*Stage 4.*—The earliest wood-destroying basidiomycetes to have entered the succession were *Corticium polygonium* and *Polyporus adustus*. Both were isolated from branch material that had been dead for 8 and 9 years, respectively. *C. polygonium* is well known both from the standpoint of the decay

it causes in mature aspen and from its frequent association with incipient staining in immature trees, particularly in the region of branch bases. It was evident from this study that dead branches had provided the most important means for this fungus to enter the heartwood. This is shown by its tendency to dominate the branch-infecting organisms in the later stages of colonization. *C. polygonium* appeared to have colonized young and small branches most frequently. For example, 94% of the branches infected by this fungus were less than 0.6 in. in diameter and 63% had less than six growth rings. The frequency of *C. polygonium*, which was higher in the mesic site (57%) than in the dry site (43%), probably reflects the greater mortality of young branches observed for trees in the mesic site. The incidence of *Polyporus adustus* was infrequent in living aspen. Its comparative rarity among all branch isolates suggests that it had little significance in the successional picture.

*Stage 5.*—It was interesting to note that *Fomes igniarius*, which causes more decay loss than any other wood-destroying fungus in aspen heartwood, appeared only rarely and always late in the succession (after 19 years). This fungus occurred in three branches, but only as lateral extensions of heartwood infections. It was isolated once from a 12-year-old stem lesion. These observations cast some doubt on the ability of this fungus to cause infections through branch stubs, and suggested that bark wounding constitutes its main avenue of entry into the heartwood of aspen.

## Discussion

Since similar temperatures and relative humidities prevail in the two sites, these factors are unlikely to play an important part in the frequency of branch infections in aspen under different growing conditions. On the other hand, the moisture content of dead branches is influenced somewhat by site, but not to the extent that it appears to affect the infection process. Only after considerable rain had fallen did branch moisture reach a level favorable for infection by fungi. Although the pattern of seasonal rainfall might be instrumental in causing periodic variations in the number of new branch infections, this would not account for local variations in the colonizing of branches by fungi.

It seems likely that the susceptibility of branches, both to breakage and infection, results from a common condition that is associated with the age and diameter of branches when they die. Young branches are more prone to infection than old branches, which suggests that either branch-invading organisms demand substances that are present in higher concentrations in young tissues (especially in the pith) for their growth, or that accumulations of growth-retarding substances occur with the formation of heartwood in older

FIG. 3. Partially embedded dead terminal (center) infected by *Cytospora* spp. and *Phoma* sp. Adjacent branches (upper and lower) uninfected.
FIG. 4. Partially embedded dead terminal (upper) infected by *Cytospora* spp. and *Phoma* sp. Adjacent branches (lower) uninfected.
FIG. 5. Fungal succession evidenced in branch stub (tagged) with *Cytospora* spp. at proximal end and *Phoma* sp. at distal end.
FIG. 6. Branch stubs depicting typical entry points for heartwood fungi.

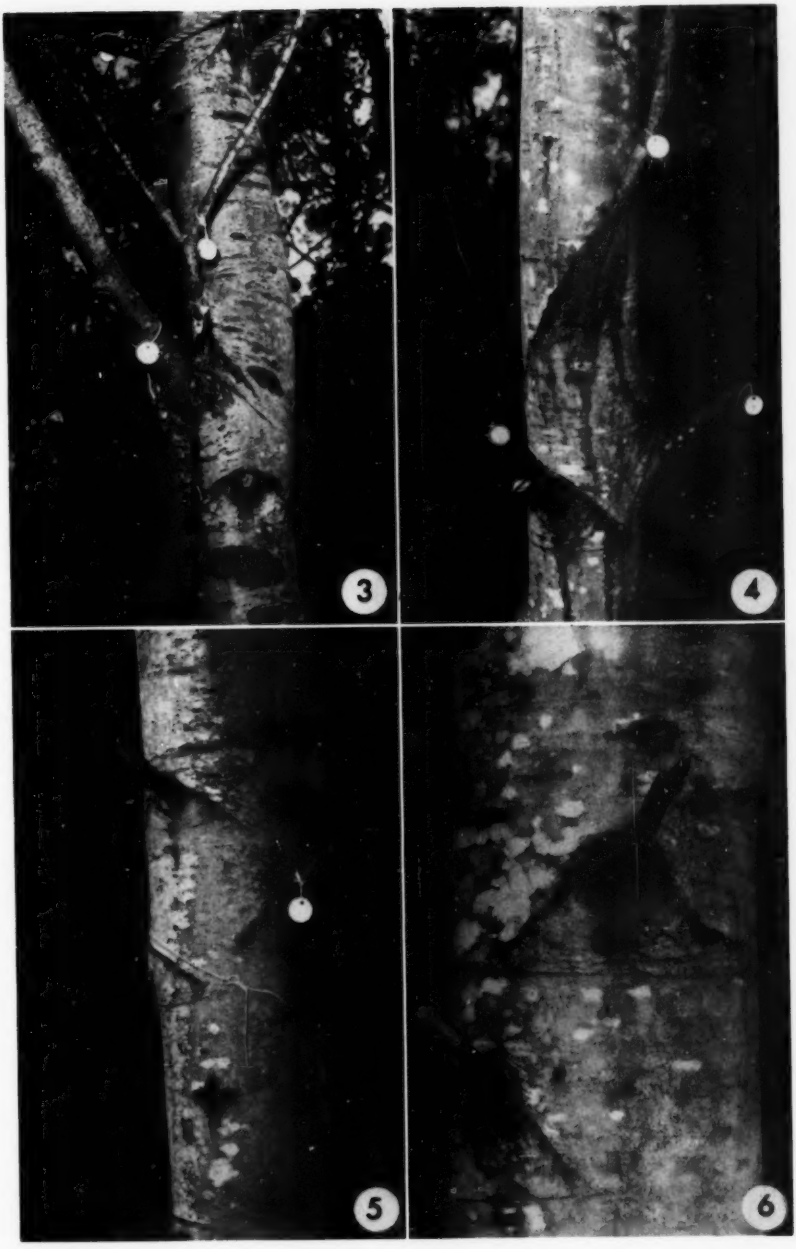


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TABLE X

Fungi and bacteria isolated from branches of aspen at different ages after their death

| Years since death of branches | No. of separate infections | | | | | | | | | No. of branches sampled |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bacteria | *Cytospora* spp. | *C. chrysosperma* | *Phoma* sp. | *Libertella* sp. | *Corticium polygonium* | *Polyporus adustus* | *Fomes igniarius* | Miscellaneous fungi | |
| 0 | *2* (Stage 1) | | | | | | | | | 5 |
| 1 | | | | | | | | | | 2 |
| 2 | | | | | | | | | | 3 |
| 3 | | | | | | | | | | 1 |
| 4 | | *1* (Stage 2) | | | | | | | | 6 |
| 5 | | 2 | | | | | | | | 11 |
| 6 | 1 | 2 | *3* | *1* | *1* (Stage 3) | | | | *4* | 16 |
| 7 | | 2 | 3 | | | | | | 4 | 12 |
| 8 | | 1 | 1 | | 1 | *1* (Stage 4) | | | 2 | 11 |
| 9 | | 1 | | | | 3 | *1* | | 5 | 15 |
| 10 | | 1 | | | | 1 | | | 2 | 11 |
| 11 | 2 | 1 | | | | 1 | 1 | | 1 | 9 |
| 12 | 2 | | 1 | 1 | | 1 | | | 2 | 14 |
| 13 | | | 1 | 1 | | | | | 2 | 13 |
| 14 | 1 | | | 2 | | 1 | | | 2 | 8 |
| 15 | | 1 | | | | 1 | | | 3 | 15 |
| 16 | | | | 2 | | 1 | | | | 12 |
| 17 | | | 1 | 1 | | | | | | 10 |
| 18 | 1 | 3 | 2 | 5 | | 2 | | | 2 | 17 |
| 19 | 1 | 3 | | | | | | (Stage 5) *1* | 1 | 8 |
| 20 | | 1 | 1 | 3 | | | | | 3 | 14 |
| 21 | | 4 | | 3 | | 1 | | | 4 | 16 |
| 22 | 1 | 1 | 1 | 2 | | | | | 3 | 16 |
| 23 | | | 1 | 1 | 1 | 1 | | 1 | | 7 |
| 24 | | 4 | 1 | 2 | 1 | 5 | | | 3 | 12 |
| 25 | | 1 | 3 | 3 | 1 | 5 | | | 3 | 11 |
| 26 | | 1 | | | | 2 | | | 3 | 8 |
| 27 | | | | 1 | | | | | 1 | 2 |
| 28 | | | | | | | | | 1 | 1 |
| 29 | | | | | | | | | | 1 |
| 30 | | | | | | | | 1 | | 1 |
| Totals | 11 | 30 | 19 | 28 | 5 | 26 | 2 | 3 | 51 | 288 |

tissues. A physical factor associated with bark thickness could also be responsible, in the sense that better protection is provided against fungi with increasing branch age. The frequent isolation of fungi from dead terminal shoots, most of which were less than 0.2 in. in diameter and not more than 2 years old at mortality, supports these possibilities since it was in extremely young tissues that the highest incidence of microorganisms occurred. In other respects, it seems unlikely that branch diameter could influence the infection process.

It is possible, therefore, in attempting to explain the higher incidence of branch infections in mesic sites, to postulate the occurrence of a greater number of small, dead branches on trees in these sites. Conceivably, such a condition could arise from an earlier branch mortality, through the suppression of twigs and branches under the denser canopy of mesic sites. Branches that die because of suppression in the more open stands in dry sites are older and larger, and are therefore more resistant to infection. If this reasoning is correct, more infections might be expected in mesic sites, despite the demonstrated capacity of trees in such sites to cover exposed branch bases more rapidly. Some evidence to support this hypothesis was obtained in the present study. The occurrence of broken branches and the occurrence of branches with less than six growth rings were compared in four trees from each site. The percentage of broken branches recorded for dry- and mesic-site trees was, respectively, 14.3 and 20.7, while the percentage of branches with less than six annual rings was 23.4 and 37.7. Experimental work to substantiate these observations with a larger sample is clearly needed, together with further studies to determine the reasons for the greater susceptibility to infection by young tissues.

The succession of organisms that is associated with the colonization of dead aspen branches features at least three successive stages before the establishment of heart-rot fungi. The preliminary invaders include bacteria (occurring also in living branches), *Cytospora* spp., *C. chrysosperma*, *Phoma* sp., and *Libertella* sp., which dominate branch infections during the 7 years immediately following mortality. It is of particular interest that the fungi which are associated with heart rot in mature aspen do not occur in branches until about eight years following their death. In attempting to explain what appeared to be a metabiotic series in the development of decay in aspen heartwood, Basham (1) has suggested that "preliminary fungi" (including *C. polygonium* and *Libertella* sp.) might alter certain properties of the substrate prior to the establishment of decay fungi, primarily *Fomes igniarius* and *Radulum casearium*. This hypothesis has gained some support in the findings of Good (6) which indicate, *inter alia*, that a lowering of pH, presumably resulting from the action of rapidly growing saprophytes, favors the germination of spores of *F. igniarius* on wound surfaces of aspen. On the basis of the present study, it seems likely that *F. igniarius* enters the succession through bark wounding, but it is quite possible that a succession of "preliminary fungi" similar to that found in branches is involved in the wound substrate. The isolation of *C. chrysosperma* from a 12-year-old lesion colonized by *F. igniarius* suggests that this may be the case.

The failure to isolate *Radulum casearium* from aspen branches that had

been dead for periods up to 30 years confirms that this fungus is mainly involved in heartwood decay of mature and overmature aspen, and is rarely encountered in trees younger than 60 years old (Basham (1), Thomas *et al.* (8)). Additional work is needed to determine the exact mode of entry of this fungus, which might explain its relatively late appearance in the successional pattern.

Artificial pruning as a practical means of reducing trunk rot in aspen is an interesting possibility, but is one that depends finally on economic and utilization considerations. The data presented here on the development of heart rot in aspen are basic to any thoughts for controlling stem-rot through pruning in this species. The results of a recently concluded survey of decay in living aspen in the Boreal Forest Region of Alberta (8) show that 89.2% of the total decay volume occurred in the trunk portion of infected trees. Of this total, 34.4% was associated with infections by *F. igniarius*, 14.2% with *R. casearium*, and 12.8% with *C. polygonium*. An additional 12.4% of the total decay volume was assigned either to *R. casearium* or *C. polygonium*, on the basis of the characteristic decay where separate identification of these fungi was uncertain. If, as the evidence at hand suggests, *R. casearium* is primarily involved in advanced decays of trees older than 60 years and generally follows the establishment of *C. polygonium* through branch stubs, measures aimed at reducing the number of infections of *C. polygonium* and shortening the cutting cycle for aspen assume immediate and practical significance. Collectively, these two fungi account for almost 40% of the total decay loss in mature aspen. It is interesting to speculate, therefore, on the amount of this loss that might be avoided by managing aspen on a 60- or 70-year rotation and by judicious pruning of the trees in the early stages, especially in stands 10 to 12 years old before *C. polygonium* is established in dead branches. It would seem that such control measures against *F. igniarius* decay might be relatively ineffective, although appropriate silvicultural practices aimed at reducing the incidence of bark wounding might achieve good results against this fungus.

## Acknowledgments

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# HYPHOMYCETES OF CONIFER LEAF LITTER

## THYSANOPHORA GEN. NOV.[1]

W. BRYCE KENDRICK


## Abstract

A new genus, *Thysanophora*, based on *Haplographium penicillioides* Roum., is proposed. Two species are placed within the genus: *Thysanophora penicillioides* (Roum.) Kendrick comb. nov. is redescribed from 32 collections and isolations, and *Thysanophora longispora* Kendrick sp. nov. is described from four collections on decaying needles of *Tsuga canadensis* and an isolation derived from one of these collections.


This paper is concerned with the fungus usually known as *Haplographium penicillioides* Fautrey, and another species congeneric with it. Hughes (1953, pp. 588–589) redescribed the type species of *Haplographium* Berk. & Br., *H. delicatum* Berk. & Br., which he has shown in culture to be the conidial state of *Hyaloscypha dematiicola* (Berk. & Br.) Nannf. He considered it to possess a much modified and very condensed *Hormodendrum*-like conidiophore head, with spores abstricted singly, accumulating in a slimy mass, and never becoming catenate. *Haplographium penicillioides*, unlike the type species, *H. delicatum*, has phialides which produce conidia in characteristic basipetal succession, forming dry chains of spores without any accompanying change in the length of the sporogenous cells.

Melin and Nannfeldt (1934, pp. 426–432) isolated a fungus which they found to be identical with *H. penicillioides*. Nannfeldt made a taxonomic reassessment of this species, and, referring to work by Mason (1933, pp. 61–63) on the genus *Haplographium*, decided that *H. penicillioides* could not adequately be classified in this genus. He also believed that no existing genus could properly accommodate this fungus, but did not propose a new one for it, and the systematic position of the species has remained unresolved up to the present.

Some of the existing confusion dates from Preuss's description of three new hyphomycetes which he had collected on decaying pine needles near Hoyerswerda, Germany. He placed them in the genus *Penicillium*, naming them *P. finitimum*, *P. flexuosum*, and *P. fuscipes*. Preuss described them as dematiaceous, and Saccardo (1886, p. 307), presumably because of this, transferred all three species to *Haplographium*.

Preuss published his rather short diagnoses of these species in Linnaea (pp. 134–136) during 1851, and this year is usually quoted as the date of valid publication for all three. The description of *P. finitimum* had, however, already been validly published as No. 1280 of Cent. XIII of Rabenhorst's Klotzschii herb. viv. mycol., which appeared in 1849. The same description was also printed in Flora (1849, p. 87).

[1]Manuscript received February 8, 1961.
Contribution No. 116 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

I have examined slides from the Herb. B[2] and Herb. FH copies of Klotzschii herb. viv. mycol., No. 1280, labelled *P. finitimum*. These show a *Hormodendrum*-like fungus which, while it answers quite well to Preuss's description, does not resemble *Haplographium penicillioides*. No illustrations accompanied the original diagnoses, and, as far as I know, none were published elsewhere. No exsiccati of *P. flexuosum* or *P. fuscipes* were issued, and the whereabouts of the type specimens, if indeed these still exist, are unknown. Thus it is at present impossible to determine whether or not either of these species bears the resemblance to *H. penicillioides* which has been suggested by several authors on the basis of their habitat and descriptions.

Roumeguère (1890) gave a description of a fungus collected by Fautrey on decaying needles of Norway spruce (*Picea abies*) as follows:

"5289. *Haplographium penicillioides* sp. n. Filaments fertiles fasciculés à la base, simples, raides, cloisonnés, noirs, la partie supérieure est hyaline et se divise en rameaux courts, dans le sens du pied, et terminés par des chapelets de spores globuleuses, allongées, incolores (L'appareil fructifère ressemble à celui d'un *penicillium*) Forêt de Charny (Côte d'Or), Automne 1889.

Sur vieilles aiguilles de *Pinus abies*. F. Fautrey."

Roumeguère distributed rather scanty type material as Fungi selecti exsiccati. n. 5289, and Nannfeldt (*in* Melin and Nannfeldt, 1934, pp. 426–432) was apparently the first to publish illustrations from this exsiccatum. I have examined the Farlow copy of Roumeguère's exsiccatum, and although, as Nannfeldt had also found, most conidiophores had been decapitated, I saw one or two intact ones which left no doubt concerning the identity of the fungus.

This, however, was not the first recorded collection of the fungus under consideration. Trail (1888) had published a "Report for 1886 on the fungi of the east of Scotland" in which he cited one of his collections on *Picea abies* as "*Rhipidocephalum abietis* g. and sp. n. On rotting needles of *Abies excelsa*." This collection is preserved in the form of a preparation labelled "*Stachylidium* [struck out] *Rhipidocephalum abietis* off decaying needles of *Abies excelsa*, Persley Den, 22/3/86. Type Spec. [scr. Trail]" (Fig. 1). Examination of this preparation has shown that it bears *H. penicillioides*. Unfortunately, Trail published no description of any sort, and *Rhipidocephalum abietis* is therefore a *nomen nudum*. Trail did not, however, forget the name *Rhipidocephalum*, and later (Trail, 1889*a*, p. 76) during a discussion of the genus *Haplographium*, he concluded erroneously that "*H. bicolor* Grove [*H. delicatum*] may also be placed in a new genus for another species of which I had already proposed the name *Rhipidocephalum abietis* before recognising the generic relationship of the two." Once more he did not, in fact, make his intended new combination. Shortly afterwards, in a "Report for 1889 on the fungi of the east of Scotland" (Trail, 1889*b*) he did make this combination, but in view of the invalid nature of the generic name, the new combination is also rendered invalid. I have seen one of Trail's slides of "*R. bicolor*" which shows the *Haplographium* state of *Hyaloscypha dematiicola*. This is not congeneric with "*Rhipidocephalum abietis*."

[2]Reference to particular herbaria is made by use of the abbreviations found in Index Herbariorum (Regnum Vegetabile, Vol. 6. 1956).

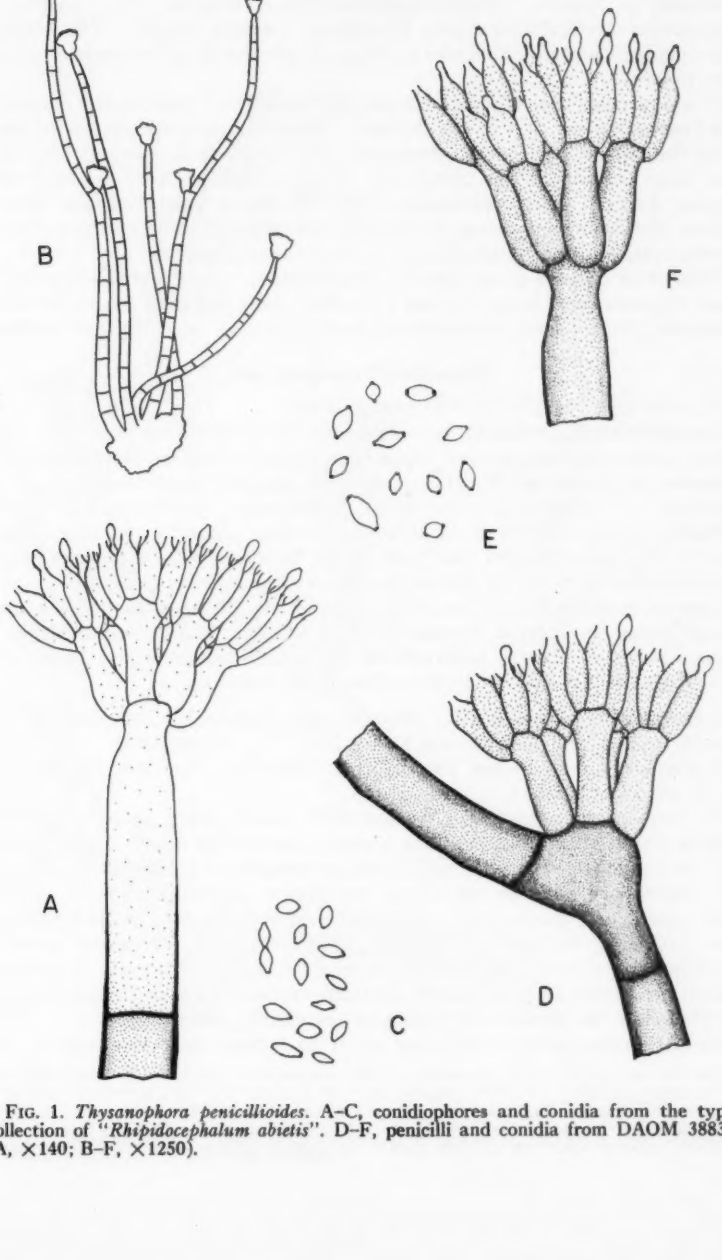


FIG. 1. *Thysanophora penicillioides*. A–C, conidiophores and conidia from the type collection of "*Rhipidocephalum abietis*". D–F, penicilli and conidia from DAOM 38838 (A, ×140; B–F, ×1250).

Later, Saccardo (1892, p. 589) published a generic diagnosis for *Rhipidocephalum* as follows: "*Rhipidocephalum* Trail. Hyphae apice basidiis ter penicillatim fasciculato-ramosis coronatae. Conidia hyalina. (*R. bicolor*)." His unhappy choice of *R. bicolor* as the type left the situation no less confused than before.

It is now clear that *Haplographium finitimum* (Pr.) Sacc. is not relevant to this discussion, and that in the absence of illustrations or specimens we cannot know the nature of either *H. flexuosum* (Pr.) Sacc. or *H. fuscipes* (Pr.) Sacc. It is also clear that of the two names given to authenticated material of our fungus, the first, *Rhipidocephalum abietis* Trail, is a *nomen nudum*, and the second, *Haplographium penicillioides* Roumeguère,[3] can contribute only its specific epithet, because the fungus is not a *Haplographium*.

I therefore propose a new genus, *Thysanophora*, to contain this species, the name *Thysanophora* being derived from 'Θυ'σανον φερουσα' or 'tassel bearer'. A redescription, based on examination of a number of collections, follows.

### *Thysanophora* gen. nov.

Fungi imperfecti, hyphomycetes saprophytici.

**Conidiophora** atro-brunnea, aggregata vel solitaria, stipite unico penicillum unum vel pluros ferente, saepe positive phototropica. **Stipites** erecti vel ascendentes interdum geniculati, simplices, septati, atrobrunnei, saepe subapicaliter et nonnunquam repetiter proliferentes, proliferatione simplice. **Penicilli** metula una vel pluribus, metula singula phialides plurimae in apice ferens. Penicilli apicaliter nati sed saepe laterescens a subapicali excrescentia stipitis. Penicilli in primis saepius a subhyalinis pallido-fusci, deinde fuscescens et nonnunquam cum stipite concolores. **Phialosporae** amerosporae siccae, natae in catenis basipetalibus, a hyalinis pallido-fuscae, leves vel minute scabrae. **Status sclerotialis** et conidiophorae saepe inveniuntur socii. **Habitat** saepius in aciculis coniferarum putridis.

***Thysanophora penicillioides*** (Roumeguère) Kendrick comb. nov.

≡*Haplographium penicillioides* Roum. Rev. Myc. 12, 68. 1890.

=*Sclerotium glauco-albidum* Desmazières. Ann. Sci. Nat. Bot. et Biol. Vég. Ser. 3. vol 16, 329. 1851.

On the natural substrate, *Thysanophora penicillioides* produces **conidiophores** which are foliicolous, dark brown, clustered or solitary, with a single stipe bearing one to many penicilli, and are sometimes positively phototropic. The **stipes** are erect or ascending, sometimes geniculate, simple, septate, dark brown, and thick-walled, but paler and with thinner walls toward the apex. Following the production of an apical penicillus, secondary growth of the stipe often occurs by means of a simple subapical proliferation. A succession of such proliferations and penicilli is often produced by a single conidiophore, and this increases the already high spore-producing potentialities of a conidiophore. A similar proliferation may occur to replace any conidiophore head

[3]The citation of Fautrey as the author of this species was introduced by Saccardo (1892), and has been accepted by all subsequent authors. Fautrey was not, however, cited as the author in the identical diagnoses given on the exsiccatum and in Revue Mycologique (1890). There appears to be no clear evidence that Fautrey did more than collect the material, and I therefore consider that the citation should be *Haplographium penicillioides* Roumeguère.

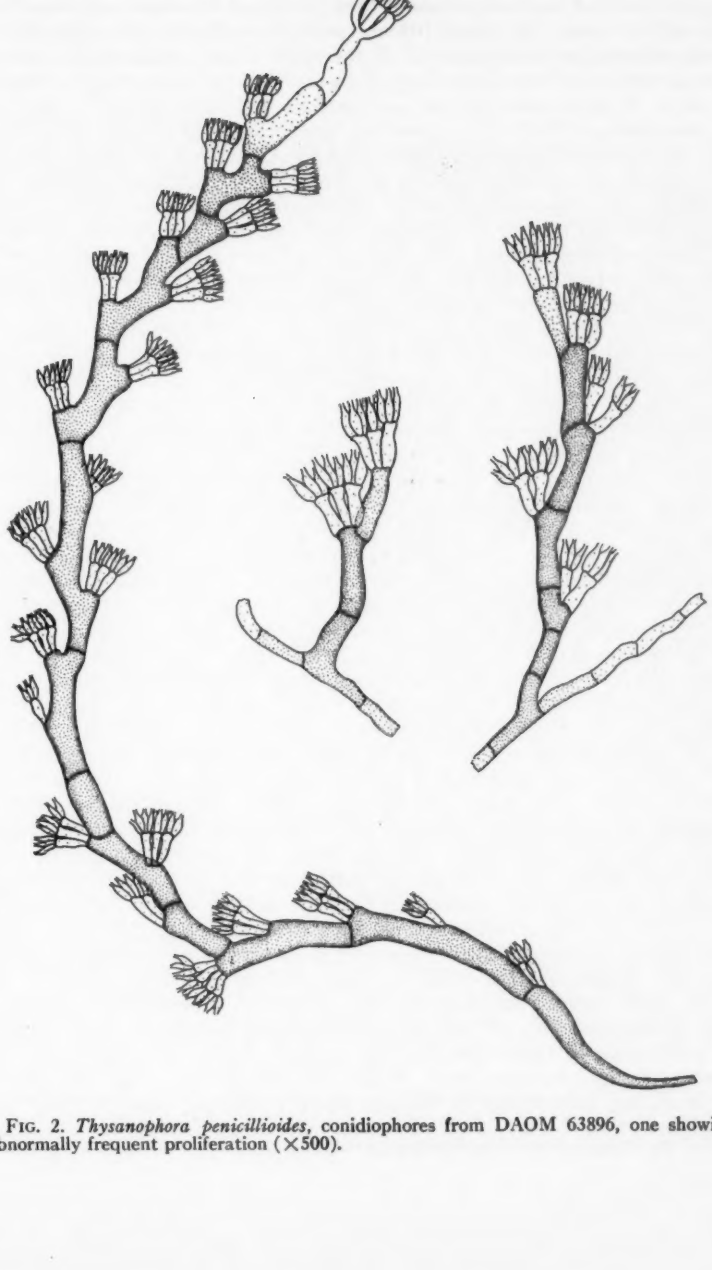

FIG. 2. *Thysanophora penicillioides*, conidiophores from DAOM 63896, one showing abnormally frequent proliferation (×500).

which has been broken off. Stipes are very variable in length, ranging up to 940 $\mu$. Their thickness ranges from 8.2 to 16.6 $\mu$ at the base, and from 3.8 to 9·6 $\mu$ at the apex. The **penicilli** are produced apically, but often become lateral following subapical growth of the stipe. They are subhyaline to pale brown at first, later often darkening, and sometimes becoming concolorous with the stipe. A single conidiophore may produce 1–5(–23) penicilli (Figs. 1, 2). The **metulae** are (2–)3–8 in a penicillus, and measure 10.8–22.6 × 3.0–8.1 $\mu$. They are borne in a parallel or slightly divergent cluster at the apex of the stipe. Each metula in turn bears a group of three to eight phialides at its apex. The **phialides** are of characteristic shape, similar to those of *Penicillium*, having a narrow neck and lacking a collarette. They measure 6.8–14.4 × 2.3–4.0 $\mu$. The **phialospores** are dry amerospores, borne in basipetal chains. They are subhyaline to pale brown, and are often minutely roughened. Ranging from subglobose to ellipsoidal in shape, they measure 1.8–6.7 × 1.4–4.0 $\mu$, and show a more or less conspicuous scar at each end. **Sclerotia** if present are superficial, subspherical, and up to 400 $\mu$ in diameter. They are translucent at first, becoming creamy white then light brown when very old. Initially they are completely invested by fine, brown, thin-walled, ramose aerial mycelium, but later slowly emerge from this integument. *T. penicillioides* is usually found on decaying needles of *Abies*, *Picea*, or *Tsuga*. The conidiophores may be sparse or very dense (Fig. 5), arising from the stomates. In *Abies* and *Tsuga* they arise almost exclusively on the lower surface of the leaf. In *Abies* and *Tsuga*, sclerotia, if present, are also usually hypophyllous, and are arranged in two irregular rows, one on each side of the midrib (Fig. 6). When occurring on *Picea*, conidiophores and sclerotia may be found on all four sides of the needles.

In culture, the colonies are quite variable in appearance and growth rate, usually, however, being of fairly restricted growth, with a diameter on malt agar at 15 days of 2.6–5.4 cm at room temperature. The margin is generally slightly diffuse and often rather irregular, being defined by immersed hyphae ranging in color through 'chaetura drab',[4] 'chaetura black', and 'fuscous black' to black. In freshly isolated cultures most of the colony is covered by a dense mat of conidiophores which range in color from 'chaetura black' to black. The length of stipes produced in culture often considerably exceeds that of those produced on the host, ranging up to 2400 $\mu$. Conidiophores produced in culture are usually more slender than those on the host, their thickness at the base ranging from 4.8 to 11.5 $\mu$ only. Stipe thickness at the apex, however, is the same as that recorded on the host, from 3.8 to 9.6 $\mu$. Production of conidia is very variable; sometimes they are sparse, visible only as a faint dustiness of the culture, while in other isolates almost the entire upper surface of the colony is covered with a powdery mass of conidia. Masses of conidia commonly appear 'grayish olive', but may also be 'light grayish olive', 'deep grayish olive', 'drab', 'citrine drab', and 'tea green'. Where aerial mycelium is found growing between or over the conidiophores (usually in older isolates), this may range in color through 'hair brown', 'drab', 'chaetura drab', and 'olive brown' to 'chaetura black'. When sclerotia appear in culture, they are almost always surrounded by floccose aerial mycelium, ranging from 'dark

[4]Colors cited from Ridgway, R. Color standards and color nomenclature, Washington 1912. Copy in the Library of the Research Branch, Canada Department of Agriculture, Ottawa.

grayish olive' to 'chaetura drab'. They are up to 450 $\mu$ in diameter, 'olive buff' to 'cinnamon buff' internally when mature, and of a hard, dry consistency. TYPE: on needles of *Picea excelsa*, Forêt de Charny, Côte d'Or, France. Autumn 1889. Coll. F. Fautrey. C. Roumeguère, Fungi selecti exsiccati n. 5289.

*Specimens Examined*

*Coniferous hosts.*—On *Abies balsamea* needles: (1) Fundy National Park, New Brunswick, August 1960, W.B.K., DAOM 71150; (2) Mabou, Cape Breton Island, Nova Scotia, August 1960, W.B.K., DAOM 71165; (3) Petawawa Forest Experiment Station, Chalk River, Ontario, September 1960, W.B.K., DAOM 71224; (4) Blue Sea Lake, Quebec, September 1960, W.B.K., DAOM 71239; (5) Paul Smith's College, New York, October 1960, W.B.K., DAOM 71328. From *Abies balsamea* butt-rot: (6) Deer Lake, Newfoundland, 1952, Redmond, as *Haplographium penicillioides* (Det. Wells), DAOM 29201. On *Abies grandis* needles: (7) Bangor, North Wales, 1959, Hayes, as *Rhipidocephalum* sp., Herb. IMI 76005, DAOM 62110. On *Abies pectinata* needles: (8) Wienerwald, Austria, 10 May 1904, von Höhnel, as *Haplographium penicillioides*, Herb. von Höhn. 1496*a*, in Herb. FH, slide DAOM 63805; (9) Wienerwald, Austria, 24 September 1904, von Höhnel, as *Haplographium penicillioides*, Herb. von Höhn. 1496*a*, in Herb. FH, slide DAOM 63806; (10) Wienerwald, Austria, 28 August 1905, von Höhnel, as *Haplographium penicillioides*, Herb. von Höhn. 1496*a*, in Herb. FH, slide DAOM 63807; (11) Wienerwald, Austria, 21 August 1906, von Höhnel, as *Haplographium finitimum*, Herb. von Höhn. 1497, in Herb. FH, slide DAOM 63809; (12) Sonntagsberg, Austria, April 1915, von Höhnel, as *Haplographium finitimum*, Herb. von Höhn. 1497, in Herb. FH, slide DAOM 63810. On *Picea abies* needles: (13) Persley Den, Scotland, 22 March 1886, Trail, as *Rhipidocephalum abietis*, Herb. Trail in Herb. ABD; (14) Forêt de Charny, Côte d'Or, France, Autumn 1889, Fautrey, as *Haplographium penicillioides*, Roumeguère, Fungi sel. exsiccati. n. 5289, in Herb. FH. On *Picea glauca* needles: (15) Fundy National Park, New Brunswick, August 1960, W.B.K., DAOM 71149; (16) Petawawa Forest Experiment Station, Chalk River, Ontario, September 1960, W.B.K., DAOM 71228. On *Picea mariana* needles: (17) Petawawa Forest Experiment Station, Chalk River, Ontario, September 1960, W.B.K., DAOM 71225; (18) Paul Smith's College, New York, October 1960, W.B.K., DAOM 71329. From *Picea omorika*: (19) West of Scotland, 1945, Dovaston, as *Haplographium penicillioides*, Herb. IMI 702, DAOM 63903. On *Picea rubens* needles: (20) Mabou, Cape Breton Island, Nova Scotia, August 1960, W.B.K., DAOM 71166. From *Picea* sp. needles: (21) St. Williams, Ontario, January 1959, Barron, DAOM 60788. On *Tsuga canadensis* needles: (22) Merivale, Ontario, October 1953, Hughes, DAOM 38838 (Fig. 1); (23) Petawawa Forest Experiment Station, Chalk River, Ontario, September 1960, W.B.K., DAOM 71232; (24) South March, Ontario, October 1960, W.B.K., DAOM 71316.

*Other sources.*—From fruit body of *Coprinus micaceus:* (25) France, October 1959, Nicot-Decaix, DAOM 64733. On old berry of *Cornus mas*: (26) Sonntagsberg, Austria, March 1918, von Höhnel, as *Haplographium finitimum* f. *fructi-*

*cola*, Herb. von Höhn. 1497, in Herb. FH, slide DAOM 63811. From forest soil: (27) Yamabe, Hokkaido, Japan, 1959, Yokota, DAOM 63910. From soil: (28) Tokyo, Japan, 1951, Tubaki (*in* Kominami, Kobayasi and Tubaki,1952), as *Haplographium chlorocephalum*, later redisposed as *Haplographium penicillioides*, DAOM 63896, Herb. IMI. 51575. On old berry of *Sorbus aucuparius*: (29) Sonntagsberg, Austria, April 1918, von Höhnel, as *Haplographium finitimum* f. *fructicola*. Herb. von Höhn. 1497, in Herb. FH, slide DAOM 63812. From ground woodpulp: (30) Iggesund, Sweden (1934), Melin & Nannfeldt, as *Haplographium penicillioides*, DAOM 63902, Herb. IMI 560A. From unknown substrate: (31) Netherlands, 1948, van Beyma, as *Scopularia tenuissima* (redisposed by Mason as *Rhipidocephalum penicillioides* (Fautrey) Mason, ined.), Herb. IMI 24802, DAOM 63894. As aerial contaminant: (32) Ottawa, Ontario, 1950, Illman, as *Haplographium penicillioides*, DAOM 29204.

From the foregoing it will be seen that *Thysanophora penicillioides* is a cosmopolitan species. In general, it seems that its geographical distribution is likely to be limited only by that of its coniferous hosts.

Von Höhnel's earlier collections (8–12) were all on fir needles, which he termed merely "Tannennädeln". Melin and Nannfeldt, however, referred to his collections as being on silver fir, presumably meaning *Abies pectinata*. Although this identification cannot easily be confirmed from the partly decayed needles of the herbarium specimens, I accept it because "Tannen-" is often used in German to refer specifically to *Abies pectinata*. Von Höhnel (1906) compared his early collections with Fautrey's material (14) and decided that they were conspecific. However, he also decided that his fungus tallied with the description of *Haplographium finitimum* (Pr.) Sacc. and applied this name to later collections. In addition, an apparent variability in the size of the spores produced by his collection in different environmental conditions persuaded him that the other two Preuss species were merely variants of *H. finitimum*. Examination of the type material of *H. finitimum* has shown that von Höhnel was wrong in identifying *H. penicillioides* with *H. finitimum*, and since he never saw specimens of the other Preuss species, his opinions concerning their identities may be disregarded. Four of von Höhnel's collections (8, 9, 12, 29) showed an apparent association between the conidial fungus and spherical, whitish sclerotia surrounded by wefts of fine, ramose, brown hyphae. Von Höhnel decided that these sclerotia were conspecific with *Sclerotium glauco-albidum* Desmazières, and suggested that they constituted part of the life history of the *Haplographium*. I have observed sclerotia in 22 of the 32 specimens examined. They may be present either on the host, or in culture, or in both circumstances. Specimens with sclerotia: 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 16, 17, 18, 19, 20, 22, 23, 24, 27, 29, 32.

I have examined the type material of *Sclerotium glauco-albidum* Desm. (Fig. 7). This exsiccatum was published in 1851 as Plantes Crypt. de France, Series 1, No. 2034, and Series 2, No. 1634, with the following description. "S. hypophyllum, minutissimum, superficiale, globoso-depressum, laeve, nitidulum, glauco-albidum, intus album; villo brunneo insidens. Hab. in pagina inferiori foliorum *Taxi*. Aestate." I have also seen two other collections labelled *Sclerotium glauco-albidum* Desm., one from Saccardo's Mycotheca

Veneta, No. 1231, collected at "Padova, in foliorum pag. inf. *Taxi baccatae*, in Horto botanico, vere 1876 (Bizzozero)" and the other from the Curtis Herbarium in Herb. FH, the name being accompanied by the single word 'Lille'.

Although no conidiophores are associated with the sclerotia in these collections, the sclerotia are, in my opinion, identical with those so often found with *Thysanophora penicillioides*. Thus despite doubts expressed by Melin and Nannfeldt (1934) concerning von Höhnel's suggested connection between sclerotia and conidiophores (the Swedish isolate of *T. penicillioides* produced no sclerotia), this connection seems to be well established.

The existence of *Sclerotium glauco-albidum* raises an unusual nomenclatural problem. Desmazières described his new species in 1851. The conidial state of what I believe to be the same imperfect fungus was not described until 1890. Since then the sclerotial and conidial states have been found many times in association with one another. Should both binomials be allowed to stand? If not, which of the two should be adopted?

There is no clear ruling in the International Code of Botanical Nomenclature on either question. However on the first question I agree with Mason (1937, pp. 73–75), who, in a discussion of pleomorphic fungi imperfecti, came to the conclusion "that each fungus considered as a fungus imperfectus is entitled to one legitimate binomial and to one only; and that all other binomials applied to the same fungus considered as a fungus imperfectus are synonyms of the legitimate binomial."

The question of which name to employ is more difficult. If the rule of priority is accepted, the specific epithet *glauco-albidum* must be chosen. This would be most undesirable, as the type specimen would then consist only of sclerotia, while the genus *Thysanophora* is characterized by features of its conidiophores and conidia, rather than by its sclerotia. In addition, conidiophores are found more often without sclerotia than vice versa. In the absence of a comprehensive ruling on this point in the International Code, I have chosen to regard the conidial state as of greater taxonomic significance than the sclerotial state (just as we regard a perfect state as of greater taxonomic significance than the conidial state), and have employed *Haplographium penicillioides* as the basionym for this taxon.

*Other Relevant Collections*

Oudemans (1902, pp. 770–771) described a fungus found by Koning on decaying pine needles at Bussum, Netherlands, as *Haplographium flexuosum*. Although no material is available, the description indicates that this collection was closely related to *T. penicillioides*.

Smith and Rea (1908) noted a hyphomycete found in 1903 on *Abies* needles at Halfmorton, Dumfriesshire, Scotland, as *Haplographium finitimum*. The illustrations show clearly that this was *T. penicillioides*.

Bainier (1908) identified a hyphomycete on needles of *Picea excelsa* as *Haplographium fuscipes*. Once more the illustrations show that this collection consisted of *T. penicillioides*.

Ten years later Smith (1918) recorded a hyphomycete on a decayed beam in an old house in Suffolk, England. She described it as possessing "a very dark, long, stout stalk, penicillioid branching at the tips, and bearing chains

of innumerable minute colourless spores massed into heads .... The spores are extremely small, subglobose or ovoid, and measure about 1 $\mu$ in diam. or 2 $\mu \times 1$ $\mu$. It resembles most nearly *Haplographium finitimum* Sacc.[5] though the spores are somewhat smaller than in that species. There were on the stalk small swellings where a previous apical head or a lateral head had been borne". Although I have been unable to trace any material of this collection, the description above, and the stated resemblance to the earlier collection, place this fungus almost certainly within *T. penicillioides*.

In the same year Bayliss Elliott (1918) employed the name *Haplographium fuscipes* for one of her collections. I have examined some of her material, and found that it is not relevant to the present study, being in fact a species of *Oidiodendron* Robak.

Measurements for collections which I have not been able to examine, but which are presumed to be *T. penicillioides* or related forms, are set out in Table I. Figures have been derived in two ways. (1) They are quoted from the original publication. (2) Where the author gave no measurements, but provided illustrations, either drawings or photographs, dimensions have been calculated from the magnifications given with these illustrations.

TABLE I

| Author | Date published | Conidia dimensions ($\mu$) | Phialides length ($\mu$) | No. of metulae | Metulae length ($\mu$) | Derivation of data |
|---|---|---|---|---|---|---|
| Oudemans | 1903 | 4 × 3.5 | 8 | 4 | 12 | (1) |
| A. L. Smith | 1908 | 3 × 1.5 | 10 | | | (1) |
| | | 1.5–2.8 × 0.8–1.5 | 6.4–9.0 | 5–6 | 11–13 | (2) |
| Bainier | 1908 | 2.8–5.6 × 2.8 | | 3–7 | 16 | (1) |
| | | 2.4–3.2 × 1.6–2.2 | 9.5–14.2 | 3–5 | 15.0–20.6 | (2) |
| A. L. Smith | 1918 | 1–2 × 1 | | | | (1) |

## *Thysanophora longispora*

I have recently collected needles of *Tsuga canadensis* bearing conidiophores of a hyphomycete which resembled *Thysanophora penicillioides* closely (Fig. 3). This collection differed, however, from the type species in several important characteristics, possessing fewer but much longer metulae, longer phialides, and very long and slender conidia which were nevertheless produced in basipetal chains from the phialides. This fungus was successfully obtained in pure culture, and as a proof of its relationship with *T. penicillioides*, it was found to produce sclerotia similar to those so often associated with that species. I have since made three further collections in which both conidiophores and sclerotia were found on the host (Fig. 8). The fungus is described below as a second species of *Thysanophora*.

*Thysanophora* ***longispora*** sp. nov.

**Conidiophora** foliicola, brunnea, aggregata vel solitaria, stipite unico, penicillum 1–5 ferente, positive phototropico. **Stipites** erecti vel ascendentes

[5]She was really referring to the fungus which she had earlier misdetermined as *H. finitimum* (Smith and Rea, 1908).

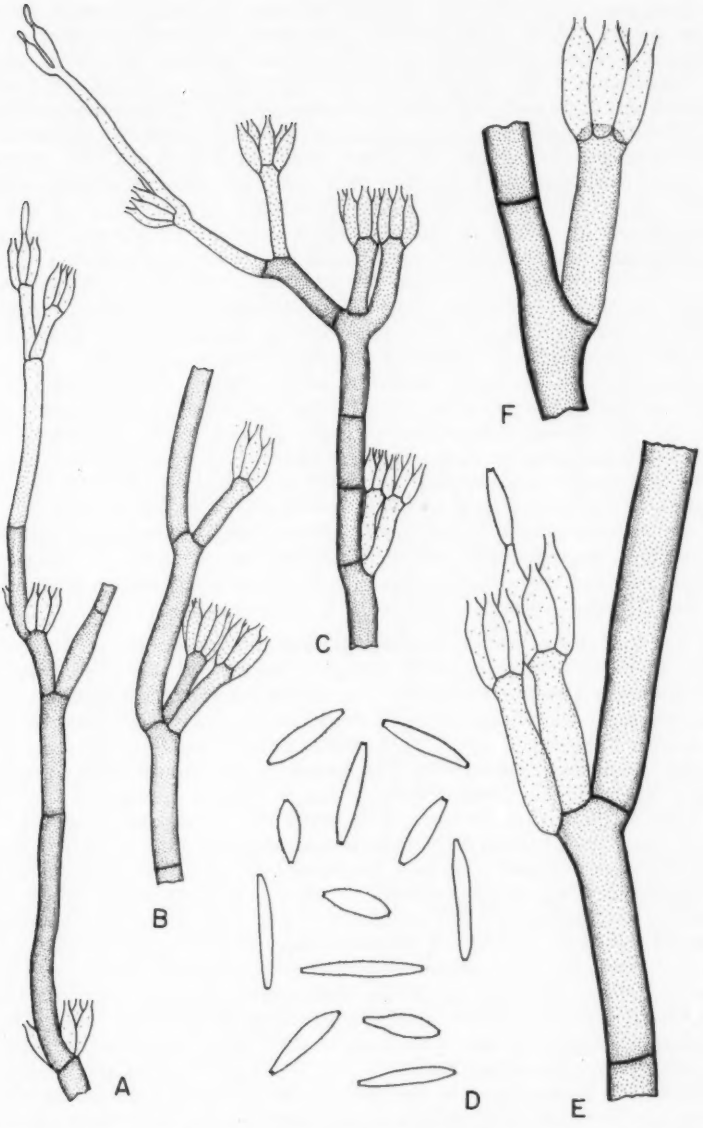


FIG. 3. *Thysanophora longispora*, conidiophores and conidia from the type collection on the host (DAOM 63073) (A–C, ×500; D–F, ×1250).

interdum geniculati, simplices, septati, brunnei, parietibus crassis; attamen versus apicem pallido fusci, parietibus tenuibus; saepe subapicaliter et nonnunquam repetiter proliferentes, proliferatione simplice. Longitudo stipitis variabilissima, ad 650 $\mu$, crassitudo ad basas 7.2–9.6 $\mu$, ad summas 4.5–6.4 $\mu$. **Penicilli** apicaliter nati, sed saepe laterescens a subapicali excrescentia stipitis. Penicilli in primis saepius a subhyalinis pallido-fusci, deinde saepe fuscescens, et nonnunquam cum stipite concolores. **Metulae** 1–4 in penicillo, paralleles vel paullum divaricatae 18.8–31.0 × 3.5–6.6 $\mu$, connatae in apice stipitis, metula singula phialides 3–7 ferens. **Phialides** 12.2–18.9 × 3.0–4.8 $\mu$, similes forma *Penicillio*, i.e. collum angustum gaudent, sed sine strophio.[6] **Phialosporae** amerosporae siccae, natae in catenis basipetalibus, a subhyalinis pallido-fuscae, gracillimes, 8.0–16.2 × 1.8–3.0 $\mu$ frequenter minute scabrae, cicatrem plusminusve conspicuam in ambas terminationes gaudentes. **Sclerotia**, si adsunt, hypophylla, superficialia, sphaeroidea ad 375 $\mu$ diam., ab subhyalinis ochroleuca. **Habitat** in aciculis *Tsugae canadensis*.

*Descriptio fungi in vitro.*—**Colonia** aetate dieum 21 crescentia finita, atra, in medio 'malt agar' dicto 2.4 cm diam., in medio 'potato dextrose agar' dicto 2.0 cm diam., marginibus paullum diffundentibus et irregularibus. **Conidiophora** positive phototropica densiter tectentia coloniam omnino, longitudo stipitis variabilissima ad 1380 $\mu$. **Metulae** 18.8–38.0 $\mu$ long. **Phialides** 12.2–22.6 $\mu$ long. **Conidia** sparsa, grisea, 8.8–27.8 × 1.8–4.0 $\mu$. Coloniae seniores mycelio sterili aereo griseo tectae, in quo sclerotia aetate suo apparuebunt nonnunquam globatim. **Sclerotia** coriacea, a sphaeroidis ovoidea, ad 500 $\mu$ diam., intus cinnamomea, extus involvuntur mycelio atrobrunneo. **Typus**: in Herb. DAOM 63073, siccus ex vitro.

On the host (Figs. 3, 8) the **conidiophores** of *T. longispora* are foliicolous, brown, clustered or solitary, with a single stipe bearing one to five penicilli, and exhibit positive phototropism. The **stipes** are erect or ascending, sometimes geniculate, simple, brown, and moderately thick-walled, but paler and thin-walled toward the apex. They often produce simple subapical proliferations, sometimes repeatedly. The length of the stipes is very variable, ranging up to 650 $\mu$. Their thickness ranges from 7.2–9.6 $\mu$ at the base, and from 4.5–6.4 $\mu$ at the apex. The **penicilli** are produced apically but often become lateral following the production of a subapical outgrowth by the stipe. They are subhyaline to pale brown at first, often darkening later and sometimes becoming concolorous with the stipe. The **metulae** number 1–4 in a penicillus, and measure 18.8–31.0 × 3.5–6.6 $\mu$. They are parallel or slightly divergent, borne in a cluster at the stipe apex, and each bears in turn at its apex 3–7 phialides. The **phialides** are of characteristic shape, resembling those of *Penicillium*, with a narrow neck, and lacking a collarette. They measure 12.2–18.9 × 3.0–4.8 $\mu$. The **phialospores** are dry amerospores, produced in basipetal chains. They may be subhyaline to pale brown, and are long and slender, measuring 8.0–16.2 × 1.8–3.0 $\mu$. They are often minutely roughened and show a more or less well-marked scar at each end. **Sclerotia** if present are hypophyllous, superficial, and spherical, up to 375 $\mu$ in diameter. In color they range from translucent 'olive buff' at first to creamy white at

[6]Strophio = collarette.

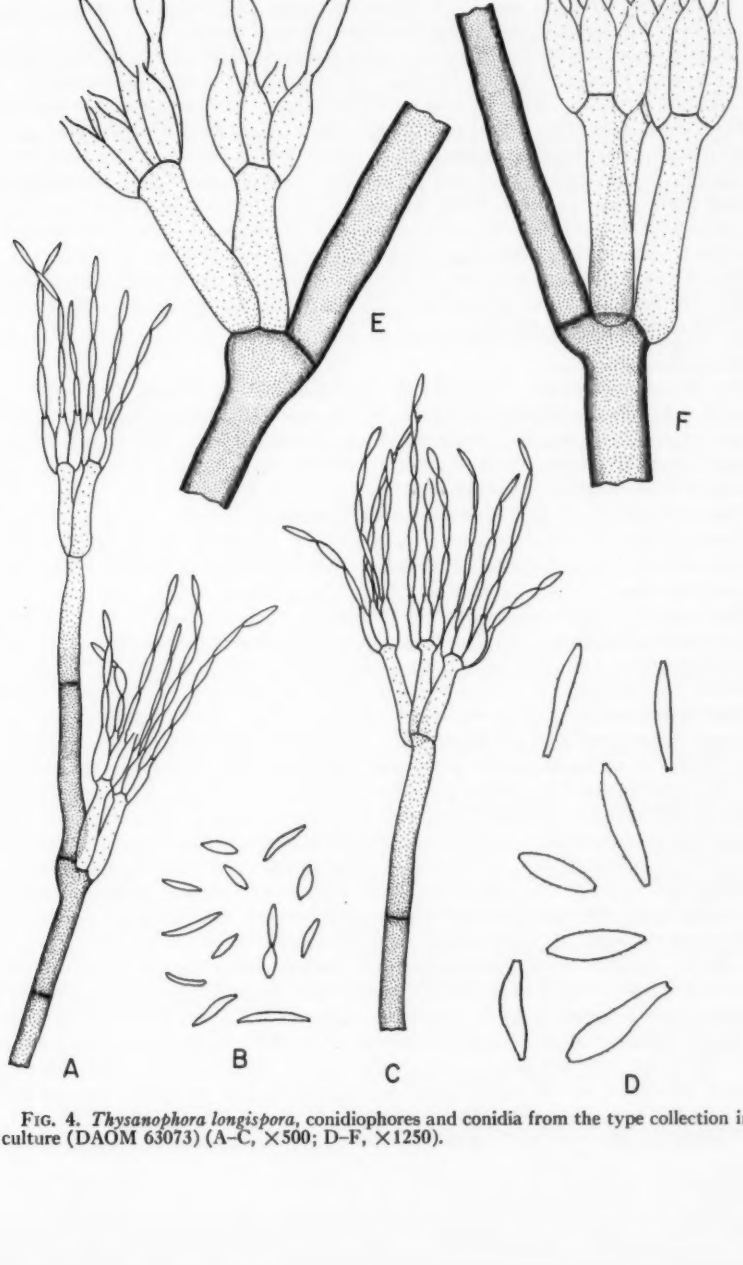


FIG. 4. *Thysanophora longispora*, conidiophores and conidia from the type collection in culture (DAOM 63073) (A–C, ×500; D–F, ×1250).

maturity. They are surrounded by fine, ramose aerial mycelium which is at first white but later darkens to 'deep grayish olive'. The fungus has so far been found only on decaying needles of hemlock (*Tsuga canadensis*).

In culture, **colonies** are of rather restricted growth, attaining a diameter of 2.4 cm after 21 days on malt extract agar, and 2.0 cm after a similar period on potato dextrose agar at room temperature. The margin is somewhat diffuse and irregular. The positively phototropic **conidiophores**, up to 1380 $\mu$ in length, form a dense mat over the whole colony. **Metulae** range in length from 18.8–38.0 $\mu$ and **phialides** from 12.2–22.6 $\mu$. **Conidia** are sparse and appear grayish. They measure 8.8–27.8 $\times$ 1.8–4.0 $\mu$ (Figs. 4, 9). In older colonies gray sterile aerial mycelium develops, within which sclerotia eventually arise. The **sclerotia** are ovoid to spherical and up to 500 $\mu$ in diameter. Externally they become invested in 'chaetura black' mycelium. Internally they are 'clay color' and of a hard, coriaceous consistency. **Type** in Herb. DAOM 63073, dried cultures.

*Specimens Examined*

On *Tsuga canadensis* needles: (1) Gatineau Park, Quebec, June 1959, W.B.K., DAOM 63073; (2) Petawawa Forest Experiment Station, Chalk River, Ontario, September 1960, W.B.K., DAOM 71227; (3) South March, Ontario, October 1960, W.B.K., DAOM 71315; (4) Bell's Corners, Ontario, October 1960, W.B.K., DAOM 71317.

The first collection of *T. longispora* (1) consisted of a few needles of *Tsuga canadensis* bearing conidiophores, but lacking sclerotia. However, cultures obtained from this collection produced both conidiophores and sclerotia. The fungus was found in abundance at Chalk River, Ontario (2), with about 5% of the recently fallen needles examined bearing fructifications of *T. longispora*. This time both conidiophores and sclerotia were present on the host (Fig. 8C). The other collections also bore both conidiophores and sclerotia.

From examination of my own collections, it appears that *Thysanophora* species infect needles soon after they have fallen to the forest floor and that the fruiting structures are produced after a period of weeks under favorable conditions, or at most within a few months. It seems probable that needles are not infected by *Thysanophora* until after they are dead, as the presence of other fungi which colonize living needles, such as *Lophodermium*, usually precludes the establishment of *Thysanophora* in the same needles. In addition, no traces of *Thysanophora* infection have been observed in living needles. The saprophytic role of *Thysanophora* seems therefore to be well-established.

Under favorable circumstances the percentage of needles infected by *Thysanophora* may be fairly high. At Petawawa, for example, of the *Tsuga canadensis* needles which had apparently fallen within the last few months, about 20% bore fructifications of *Thysanophora*. About three-quarters of these needles bore *T. penicillioides*, the other quarter, *T. longispora*. Occasionally both species were found on a single needle (Fig. 8D). Colonization may range from slight to very extensive, and single conifer needles have been observed to bear as many as 500 conidiophores, and up to 150 sclerotia, though half that number is a more usual figure (Figs. 5, 6).

Canadian collections of *T. penicillioides* on *Abies balsamea* leaf litter show a

much lower incidence of the fungus, which usually occurs on about one needle in a hundred. In one locality where *Abies* was growing in a mixed stand with *Picea*, about one in two hundred of the fallen *Picea* needles had become colonized. When the *Picea* was growing in a pure stand, the incidence of the fungus was much lower, perhaps one needle in a thousand bearing fructifications.

Examination of litter derived from other conifers found in eastern Canada has failed to reveal any infection by a *Thysanophora* species, but it is to be expected that the host range of this genus will be extended by future observations in other parts of North America.

## Acknowledgments

I would like to thank Dr. S. J. Hughes and Dr. L. K. Weresub for many helpful discussions during this study, and for reading the manuscript, which also owes much to the constructive suggestions offered by Dr. J. W. Groves and Dr. R. Macrae.

I am also indebted to Dr. M. B. Ellis and Mr. J. J. Elphick of the Commonwealth Mycological Institute for some invaluable specimens; to Mrs. M. M. Lamb of the Farlow Herbarium for the loan of numerous specimens in her keeping, to Mrs. A. H. Sommerville of the University of Aberdeen for the loan of material from Herb. Trail, and to Mrs. F. L. Balfour-Browne of the British Museum (Natural History) for allowing me to examine some of Bayliss-Elliott's material.

Dr. B. Boivin kindly translated the diagnoses into Latin.

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## EXPLANATION OF FIGURES

FIG. 5. *Thysanophora penicillioides*, dense growth of conidiophores on needle of *Abies pectinata* (DAOM 63809). A, in side view (×32); B, seen from above (×32).

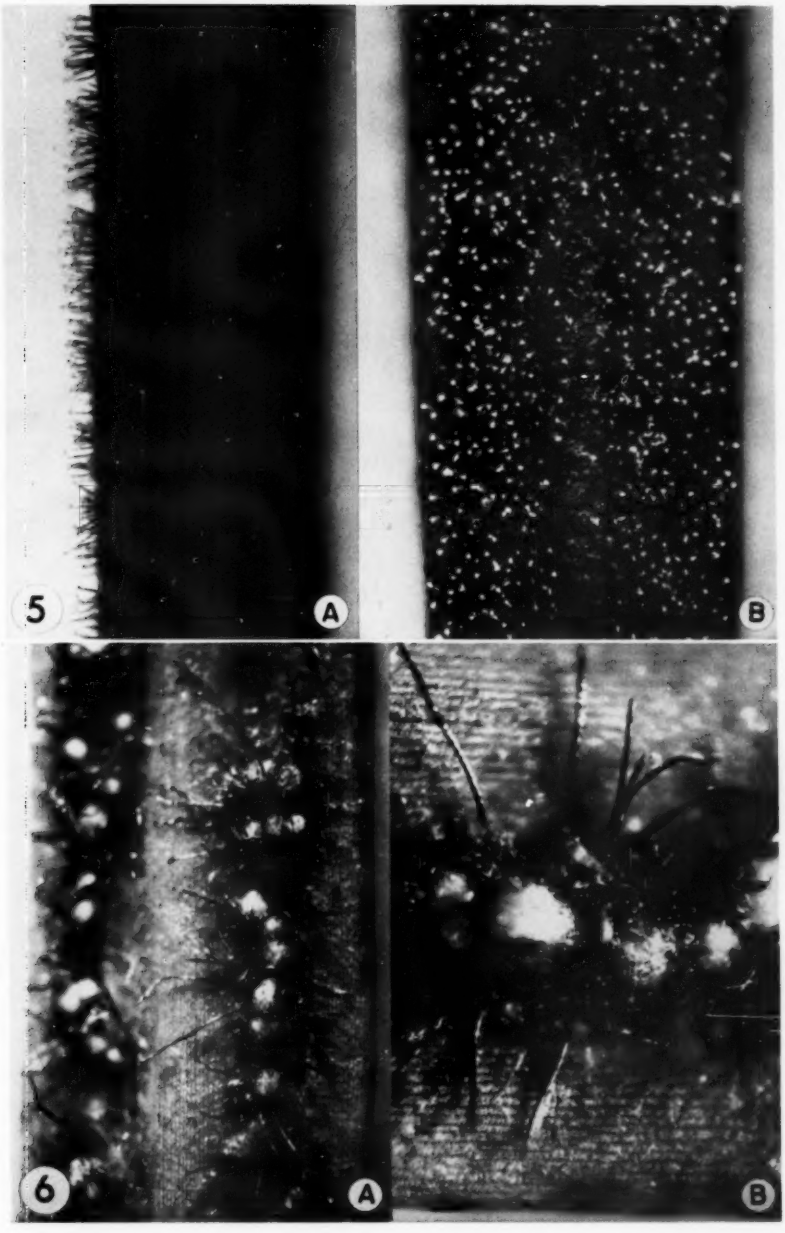
FIG. 6. *Thysanophora penicillioides*, numerous sclerotia and conidiophores on needle of *Abies pectinata* (DAOM 63810) (A, ×32; B, ×86).

FIG. 7. "*Sclerotium glauco-albidum*", sclerotia on needles of *Taxus baccata* from the type collection. A, Plantes Crypt. de France. Ser. 1. No. 2034 (×17); B, Ser. 2. No. 1634 (×17).

FIG. 8. *Thysanophora longispora* and *T. penicillioides*. A, *T. longispora*, cluster of conidiophores from needle of *Tsuga canadensis* (DAOM 71227); phase contrast (×100). B, *T. longispora*, conidiophore from culture (DAOM 63073) (×370). C, *T. longispora*, conidiophores and sclerotia from needles of *Tsuga canadensis* (DAOM 71227) (×100). D, *T. longispora* and *T. penicillioides*, conidiophores found growing together on needles of *Tsuga canadensis* (×370).

FIG. 9. *Thysanophora longispora* (DAOM 63073). A–B, conidia from culture; phase contrast (×900). C–D, penicilli from culture; phase contrast (×900).

NOTE: Figs. 5–9 follow.

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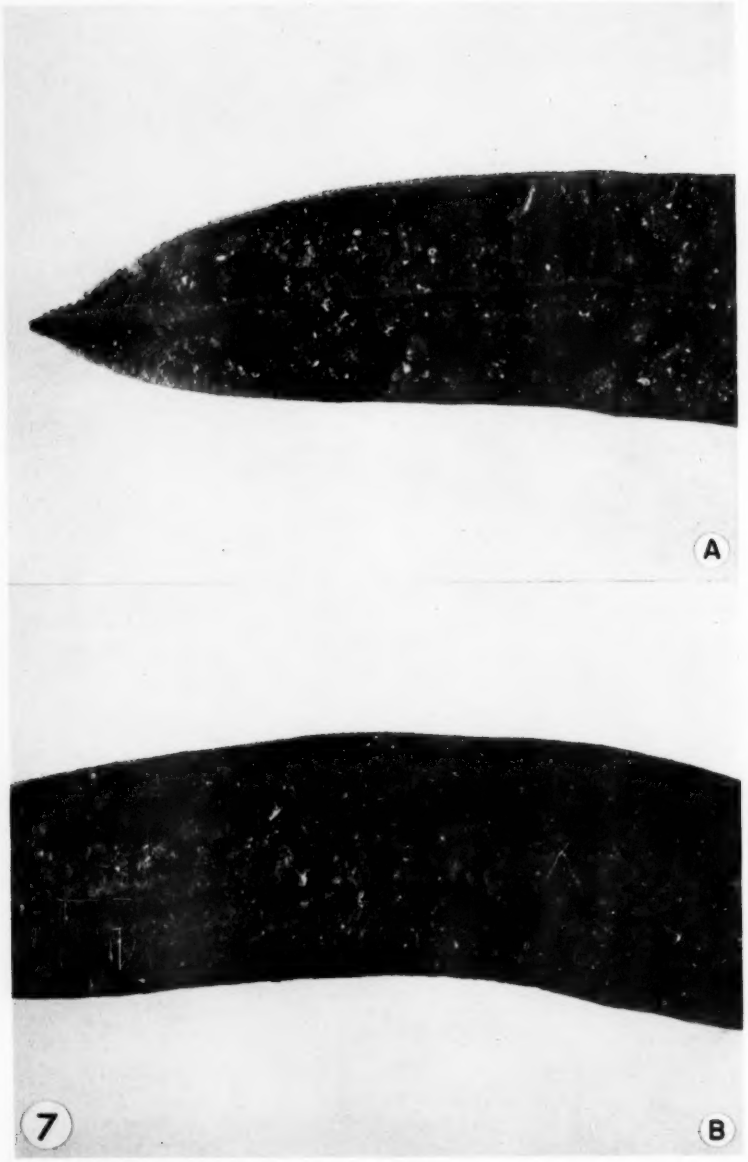


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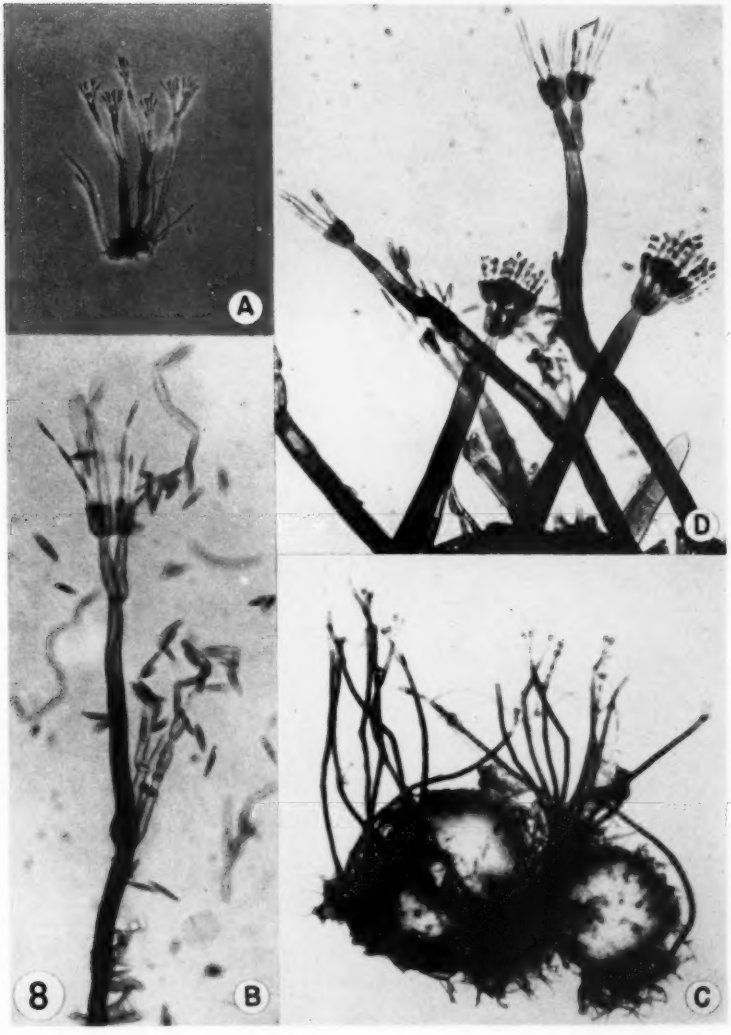


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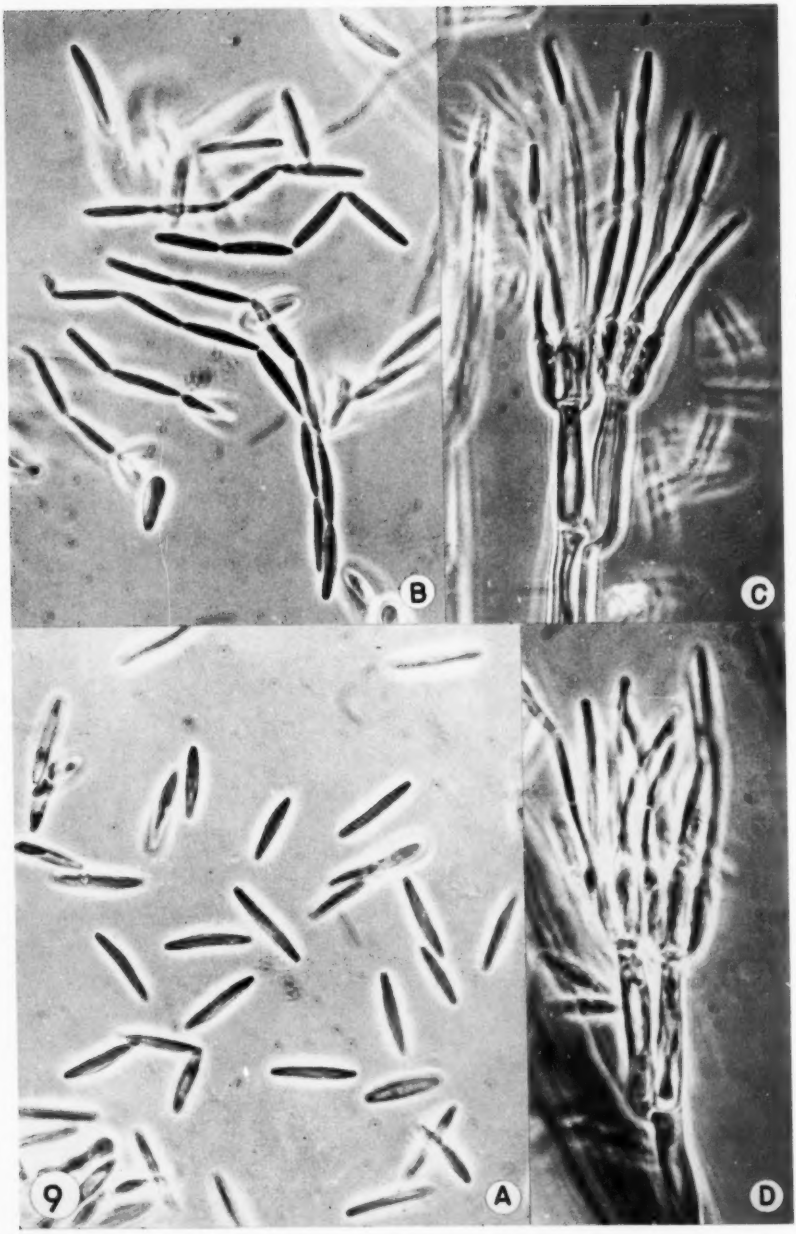


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# HYPHOMYCETES OF CONIFER LEAF LITTER

## HORMODENDRUM STAUROPHORUM SP. NOV.[1]

W. BRYCE KENDRICK


### Abstract

*Hormodendrum staurophorum* sp. nov., a pleomorphic dematiaceous hyphomycete isolated from fallen needles of *Pinus sylvestris*, is described and illustrated.


The fungus described here was isolated twice during a study of the microfungal succession associated with decaying leaf litter of *Pinus sylvestris*. Both cultures were obtained from recently fallen needles sampled from the surface litter, or L layer, of the organic horizon at Delamere Forest, Cheshire, England (Kendrick 1959). The needles had been rigorously washed in many changes of detergent and sterile water to remove surface-borne fungal spores prior to plating on acidified malt agar.

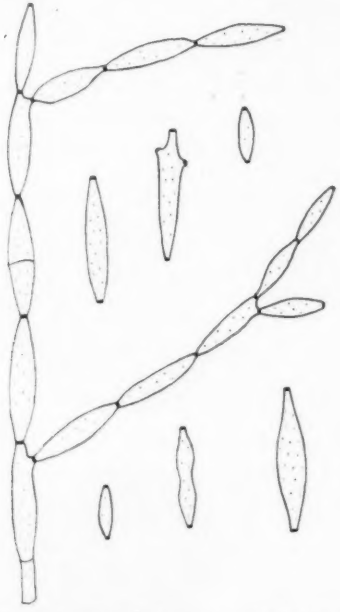

FIG. 1. *Hormodendrum staurophorum*: conidia from culture, ×1500.

[1]Manuscript received February 8, 1961.
Joint contribution from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Canada (Contribution No. 123), and the Botany Department, University of Liverpool, England.

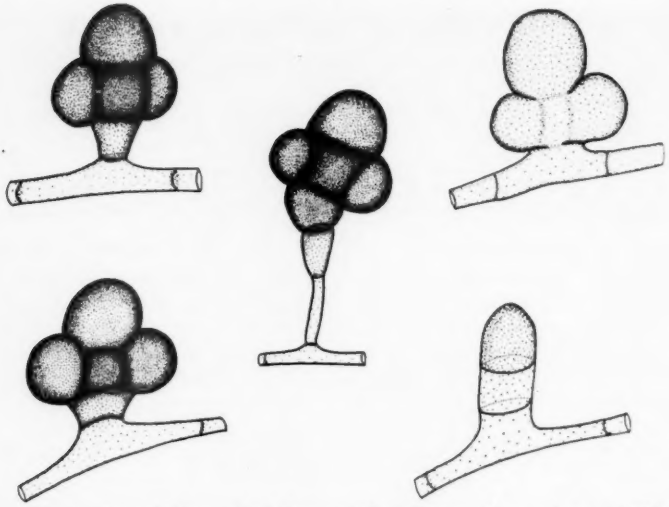

FIG. 2. *Hormodendrum staurophorum*: chlamydospores from culture, ×1500.

On malt agar, colonies are spreading and effuse. Aerial mycelium is velvety in texture, and light brown in color, while immersed mycelium is a rich brown. The mycelium is composed of branched, septate, brown hyphae which are 1.4–4.0 $\mu$ in width. The conidia (Figs. 1, 5) are inconspicuous, developing acropetally in dry, branched chains from aerial hyphae. They are non-septate, pale brown, and more or less fusiform, measuring 5.2–18.0 × 1.4–2.6 $\mu$; each end is truncate and a lateral truncate outgrowth is sometimes found near the apex of the conidum where a new branch of the spore chain has been initiated.

The very characteristic, darkly pigmented chlamydospores (Figs. 2, 3, 5) are produced abundantly on the immersed mycelium. They are cruciform, being made up usually of five cells; a truncate obconical basal cell somewhat less heavily pigmented than the others, a more or less cubical central cell which protrudes on both sides from the axial plane of the spore, two hemispherical lateral cells situated on opposite sides of the central cell, and a larger hemispherical apical cell. The entire chlamydospore is quite constant in its dimensions, being 12.5–16.8 $\mu$ long, 12.5–15.0 $\mu$ wide, and 6.8–8.2 $\mu$ thick. In older colonies, the chlamydospores are occasionally slightly roughened. They may be sessile, or are borne on an auxiliary cell or at the apex of a hypha. This unique chlamydospore, resembling the dictyospore of some species of *Monodictys* (Hughes 1958), is the feature which justifies the erection of a new species for these isolates.

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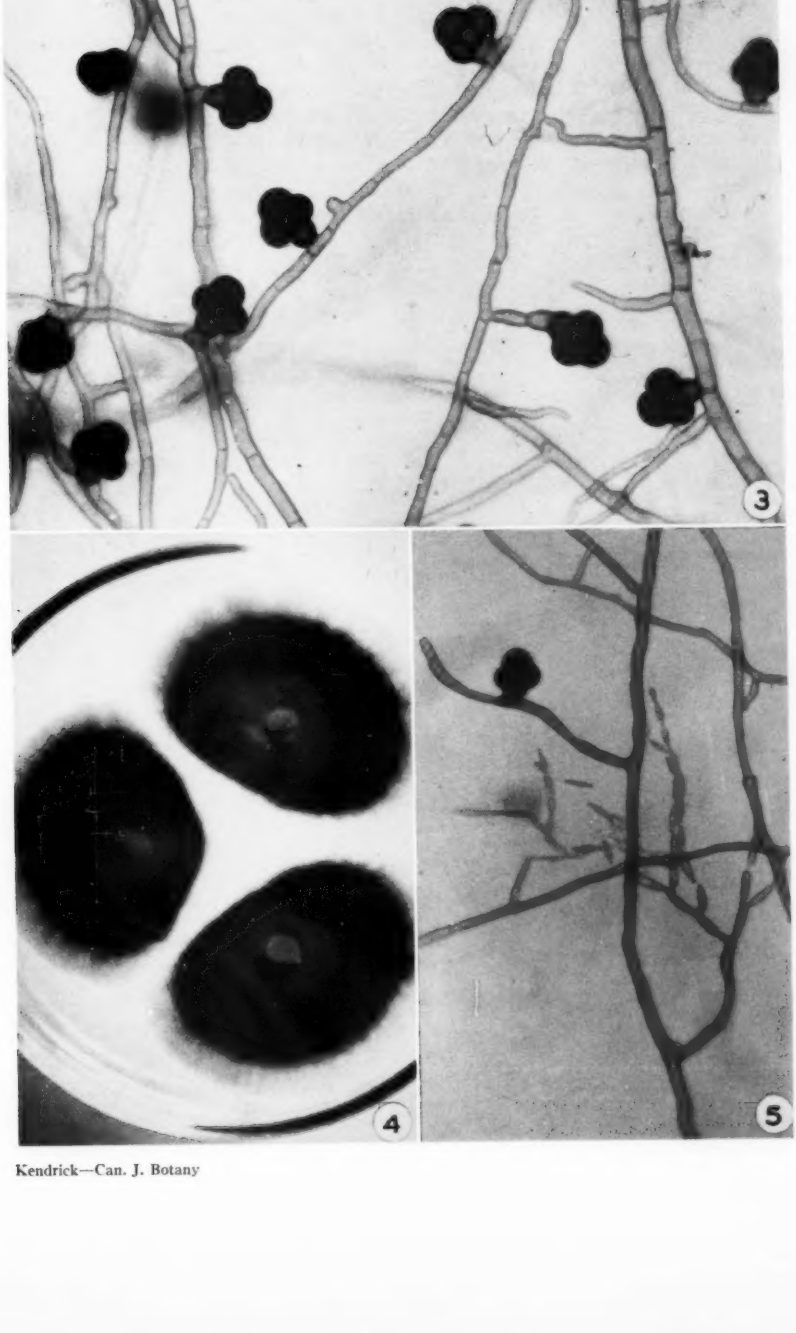
FIG. 3. *Hormodendrum staurophorum*: mycelium bearing chlamydospores, ×550.
FIG. 4. *Hormodendrum staurophorum*: colonies on malt agar at 14 days, natural size.
FIG. 5. *Hormodendrum staurophorum*: conidia and a chlamydospore developed from the same hypha, ×550.

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### *Hormodendrum staurophorum* sp. nov.

Fungi imperfecti, hyphomycetes saprophytici.

Ex culturis in agaro "malt" dicto descripta.

Coloniae radiantes, effusae. Mycelium aerium velutinum, pallide fuscum. Mycelium immersum brunneum. Hyphae ramosae, septatae, fuscae, 1.5–4.0 $\mu$ crassae. Conidia non-septata, dilute fusca, plusminusve fusiformia, 5.2–18.0 $\times$ 1.4–2.6 $\mu$, in utraque terminatione truncata, interdum versus apicem cum enatione truncata laterali; acropetalim in catenas siccas ramosas, ex hyphis aeriis oriunda. Chlamydosporae numerosae, saepe immersae, solitariae, atrobrunneae, parietibus crassis, cruciformiae in 2 dimensionibus, e cellulis 5 compositae—cellula basali obconicotruncata, cellula centrale plusminusve cubiforme, 2 cellulis hemisphaericis parvis lateralibus, atque cellula majore hemisphaerica apicali—in mycelio sessiles, vel cum cellula auxiliare, vel in hyphae apice; 12.5–16.8 $\mu$ longae, 12.5–15.0 $\mu$ latae, 6.8–8.2 $\mu$ crassae; in coloniis maturis interdum subasperae.

Habitat: ex foliis emortuis *Pini sylvestris*, Delamere, Cheshire, Anglia isolatum.

Typus: in Herbarium DAOM 60786, siccus ex vitro.

The specific epithet has been derived from the Greek '*Σταυρός φέρον*' meaning 'Cross bearer', in preference to the Latin 'cruciferum' of the same meaning, which was thought to be potentially misleading.

I am much obliged to Dr. V. H. Heywood for correcting the Latin diagnosis.

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# A REVISION OF THE GENUS PETRIELLA[1]

G. L. BARRON,[2] R. F. CAIN,[3] AND J. C. GILMAN[4]

## Abstract

A reappraisal is made of the genus *Petriella* Curzi. The type species, *Petriella asymmetrica*, is found to be identical with a species previously described as *Microascus sordidus* Zukal. The latter species is transferred to *Petriella* as a new combination, and one new species, *P. guttulata*, is described. A synopsis of the genus, with five species, is presented. The additional three species are *P. setifera*, *P. lindforsii*, and *P. boulangeri*.

## Introduction

The genus *Petriella* was erected by Curzi (1930) to include four species, with *P. asymmetrica* as the type. Curzi describes the ascocarp of *P. asymmetrica* as being perithecia-like, black and carbonaceous, with a well-developed neck and ostiolar region. The asci, distributed at all levels within the centrum, are evanescent, breaking down at maturity to release the reddish-brown ascospores into the central cavity. The ascospores, plano-convex to concavo-convex in side view, are extruded through the ostiole in the form of a long cirrhus.

In its generic characters *Petriella* closely resembles the genus *Microascus*, which had previously been erected by Zukal (1885) to include a single species *M. longirostris*. Zukal (1890) also described a second species, *M. sordidus*, which he regarded as being substantially the same as *M. longirostris* but differing in certain ascosporic and perithecial characters. *Microascus* was revised by Curzi (1931), who included there a number of species previously disposed in other genera.

*Petriella* and *Microascus* have the following characters in common: (1) the ascocarps are perithecia-like with a well-marked ostiole; (2) the asci are distributed at all levels within the centrum and are evanescent; (3) the ascospores are asymmetric in the majority of species; (4) at maturity of the ascocarp, the ascospores are extruded either in the form of a long cirrhus or as a gelatinous ball at the mouth of the ostiole, depending on environmental conditions.

In erecting *Petriella*, Curzi noted these similarities to *Microascus* but nevertheless regarded the two genera as distinct, separating them principally on the basis of the hairy condition of the perithecium in *Petriella*. Curzi observed that hairs were typically lacking in *Microascus* and, where present, he regarded them as being sparing in occurrence and rhizoidal in function. While Curzi was undoubtedly correct in maintaining the two groups as distinct genera, his separation on the basis of the hairy condition of the perithe-

[1]Manuscript received February 28, 1961.
Contribution from Department of Botany, Iowa State College, Ames, Iowa, and Department of Botany, University of Toronto, Toronto, Ontario.
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[3]Department of Botany, University of Toronto.
[4]Department of Botany, Iowa State College.

cium was not justified since the distinction is not absolute, as he implied, but rather one of degree. Barron (1958) has shown that a number of isolates recognized as being typical *Microascus* may show an abundant development of hairs on the perithecial body and neck and may even have an ostiolar fringe reminiscent of certain species of *Ophiostoma*. He also pointed out that the hairy character is considerably influenced by environment and is, therefore, an undesirable character for taxonomic purposes in this case.

*Petriella*, however, can be distinguished from *Microascus* by other criteria, particularly those associated with ascospores, conidial stages, and, to some extent, cultural morphology. In *Petriella* the ascospores are much larger and bulkier, averaging 9–10 $\mu$ long by 4–6 $\mu$ broad, and are usually red-brown in color. In *Microascus* species, with the exception of *M. schumacheri* and *M. stysanophorus*, the ascospores are 4–7 $\mu$ (rarely 8 $\mu$) long and are straw-colored. At least four and probably all five species of *Petriella* possess both *Graphium* (*Sporocybe*) and *Sporotrichum* (Barron 1958) as the conidial stages. In *Microascus*, on the other hand, *Scopulariopsis* is the conidial stage of all species so far connected, with the single exception of *M. stysanophorus*, which has both *Stysanus* and *Sporotrichum* conidial forms (Douget 1957).

While *M. shumacheri* and *M. stysanophorus* have ascospores in a similar range to *Petriella* regarding length of spore, they are somewhat narrower and have the straw color typical of *Microascus*. Culturally both of these species show a restricted growth habit unlike the spreading habit of *Petriella* species, and neither possesses a *Graphium* conidial stage. *M. shumacheri* has no known conidial stage and, as previously mentioned, *M. stysanophorus* has both *Sporotrichum* and *Stysanus* as its conidial stages.

Thus it can be seen that *M. stysanophorus* seems to be somewhat intermediate between *Microascus* and *Petriella* in its disposition but seems to be much more closely related to *Microascus* and is, therefore, included in that genus. In this the writers are in agreement with Curzi, who transferred it from *Melanospora*, where it had been placed by Mattirolo (1886).

The type species of *Petriella*, viz. *P. asymmetrica*, is of particular interest in its obvious similarities to *M. sordidus* of Zukal. The ascospores of the two species correspond almost exactly in shape and size with *P. asymmetrica*, measuring 8–10 by 3·7–5·5 by 5·0–6·2 $\mu$ while the ascospores of *M. sordidus* as measured by Zukal are 9·0–9·5 $\mu$ long by 5–6 $\mu$ broad. In both species the ascospores are red-brown in color and plano-convex to slightly concavo-convex in shape (Figs. 1, 12). Moreover, the perithecia of *P. asymmetrica* are papillate to long-necked (Fig. 10) agreeing very well with Zukal's figures and descriptions of *M. sordidus* (Fig. 1). Curzi described his species as having two conidial stages, *Sporotrichum* and *Sporocybe* (*Graphium*). Zukal, on the other hand, did not associate his isolate with a conidial form; this was probably due to the fact that Zukal described his species from the original substrate and apparently never obtained it in pure culture.

From a study of the type culture of *P. asymmetrica* and comparison of it with Zukal's figures and descriptions of *M. sordidus* (Fig. 1), there is little doubt that the two are the same fungus. *M. sordidus* of Zukal, however, is quite distinct from the other species of *Microascus* on the basis of both ascospore characters and conidial stages and Curzi was correct in assigning his

isolate to a new genus. *M. sordidus* should, therefore, be transferred to *Petriella* as *P. sordida* comb. nov. with *P. asymmetrica* Curzi as a synonym.

There remains some confusion concerning the two species *P. boulangeri* and *P. lindforsii*. Boulanger (1896) redescribed what he thought to be a new isolate of *Chaetomium cuniculorum* Fuckel with associated conidial stages *Sporotrichum* and *Graphium*. Lindfors (1920) found a second isolate which he regarded as identical with *C. cuniculorum* sensu Boulanger; however, he considered that Boulanger was in error in assigning this species to *C. cuniculorum* Fuckel and he renamed it *Chaetomium boulangeri*. Curzi pointed out correctly that both *C. cuniculorum* sensu Boulanger and *C. boulangeri* Lind. were quite distinct species and, moreover, neither of them belonged properly in *Chaetomium* but rather in his new genus *Petriella*. Curzi reclassified the isolate of Boulanger as *P. boulangeri* and that of Lindfors as *P. lindforsii*. Since there is some doubt about which isolate Lindfors had in mind in his final analysis of *C. boulangeri* it would seem appropriate to consider this name as a *nomen confusum*.

The genus *Petriella* comprises five species as follows: *P. sordida* (Zukal) Barron and Gilman, *P. guttulata* Barron and Cain, *P. setifera* (Sch.) Curzi, *P. lindforsii* Curzi, and *P. boulangeri* Curzi.

## Genus Description

Perithecia separate, without stroma, small, black, subglobose to pyriform, with single cavity, provided with a neck of variable length and with circular ostiole at apex. Hairs usually present on neck and upper part of perithecium. Asci subglobose to short clavate, eight-spored, without apical structure for ascospore dehiscence, irregularly disposed, evanescent, and breaking down at maturity to free the ascospores in the perithecial cavity. Ascospores red-brown, about $10 \times 5$ $\mu$, asymmetric with reference to axis through poles, somewhat prolate, flattened from two opposite sides, extruded in long cirrhus or globular mass (under condition of greater moisture). Conidia produced by most species, hyaline or faintly colored, one-celled, not on phialides but developed singly on denticles from sporogenous cells which are scattered on aerial hyphae (*Sporotrichum*) or forming a globose head at apex of elongated, cylindrical, brownish coremium (*Graphium* or *Sporocybe*).

### Key to Species

1. Ascospores plano-convex to concavo-convex ..... 2
   2. Ascospores with numerous small oil droplets, perithecia short-necked ..... *P. sordida*
   2. Ascospores with a few large oil droplets, perithecia papillate and extremely setose ..... *P. guttulata*
1. Ascospores ovoid or asymmetrically convex ..... 3
   3. Ascospores asymmetrically convex ..... *P. setifera*
   3. Ascospores ovoid ..... 4
      4. Ascospores with pointed ends ..... *P. lindforsii*
      4. Ascospores with rounded ends ..... *P. boulangeri*

## Species Descriptions

**Petriella sordida** (Zukal) Barron and Gilman comb. nov. (Figs. 1, 10, 12)
Basinym: *Microascus sordidus* Zukal. Ber. deuts. bot. Gesell. 8: 295–303. 1890.
Syn.: *Petriella asymmetrica* Curzi. Boll. Staz. Pat. veg. Roma, 10: 380–423. 1930.

Perithecia black, carbonaceous, papillate to long-necked, with spherical base, 200–400 $\mu$ in diameter; neck frequently half as long as diameter of base, sometimes longer and contorted, up to 400 $\mu$; perithecia and neck covered with scattered hairs; hairs short and straight on neck, longer and undulate on base, septate, brown-pigmented, smooth or with wart-like protuberances, up to 100 $\mu$ long; asci subglobose to ovoid, frequently clavate, sessile or with a well-developed foot, 18–28 $\times$ 11–19 $\mu$; ascospores plano-convex to slightly concavo-convex, 4.5–5.5 $\times$ 8.5–10.5 $\mu$, red-brown, containing numerous small oil droplets, extruded in a long dark-brown cirrhus.

Conidial stages *Sporotrichum* and *Graphium*; *Sporotrichum* conidial stage arising first; conidiophores originating from the vegetative hyphae or hyphal 'ropes', simple, long, slender, tapering, bearing conidia singly and acrogenously; conidiophores and conidia hyaline, conidia mostly pyriform, sometimes wedge-shaped or long-cylindric, tapering to a truncate base, rounded at the apical end, 3.5–5.5 $\times$ 4.5–8.5 $\mu$. *Graphium* stage soon in evidence; vegetative hyphae grow perpendicular to the substrate, anastomose and corticate to form coremia; coremia dark-brown pigmented, up to 1 mm high and 200 $\mu$ broad, sterile hyphae branching at the top to form a sporiferous head; conidia arising as *Sporotrichum* type but are released in a mucous material which appears as large gelatinous balls at the top of the coremia; gelatinous heads at first hyaline, later becoming violaceous and finally sooty in age; conidia measure 2.5–4.5 $\times$ 8–14 $\mu$, usually long cylindric, sometimes wedge-shaped or pyriform.

*Collections* were isolated from soil, Colorado, U.S.A., by L. W. Durell. From pepper-seed, Ottawa, Ontario, by J. W. Groves December 28, 1944 (TRTC 33042). From soybean seed, Fort Gary, Manitoba, by J. W. Groves, April 10, 1943 (TRTC 33041). From seed, Ottawa, Ontario, by R. F. Cain, June 29, 1955 (TRTC 33032). From forest nursery soil, Ampthill, Bedfordshire, England, by J. H. Warcup, August 1950 (TRTC 33063). From rat dung, Edwards County, Kansas, by T. E. Brooks, November 29, 1947 (1019). From debris, Cole County, Missouri, by T. E. Brooks, December 30, 1957 (1480). From decayed *Pyrus communis* leaves by C. Curzi, Italy (per C. B. S.). From dung in moist chamber, from Loon Bay, Lake Timagami, Ontario, by R. F. Cain, February, 1950 (TRTC 33018).

Colonies reach a diameter of 4–6 cm in 4 weeks on potato dextrose agar (P.D.A.). At first white, colonies turn greyish in the central portions with the production of the *Sporotrichum* conidial phase. Colonies have a raised button in the center with radial wrinkling of the agar. A clear exudate is produced in droplets scattered over the conidia-bearing regions. The *Graphium* stage may be largely suppressed, in which case the colonies remain greyish. In some isolates the *Graphium* stage is produced abundantly with coalescing of the mucous heads to give a black, gelatinous appearance over much of the colony. Perithecia are produced in abundance in the aerial mycelium and on the agar, frequently around the perimeter in older colonies. Mason (1941) used the name *Sporocybe curziana* Mason for the conidial state of this fungus.

This species is close to *P. guttulata* but differs from it in having a sparingly hairy, flask-shaped perithecium. It is also distinct in having ascospores with numerous small oil droplets.

PLATE I

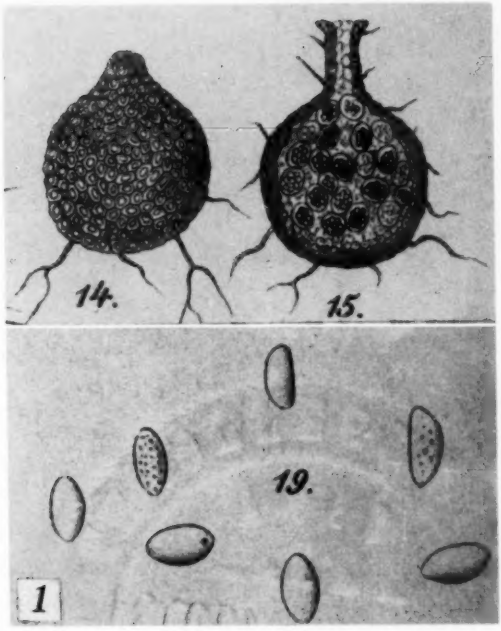
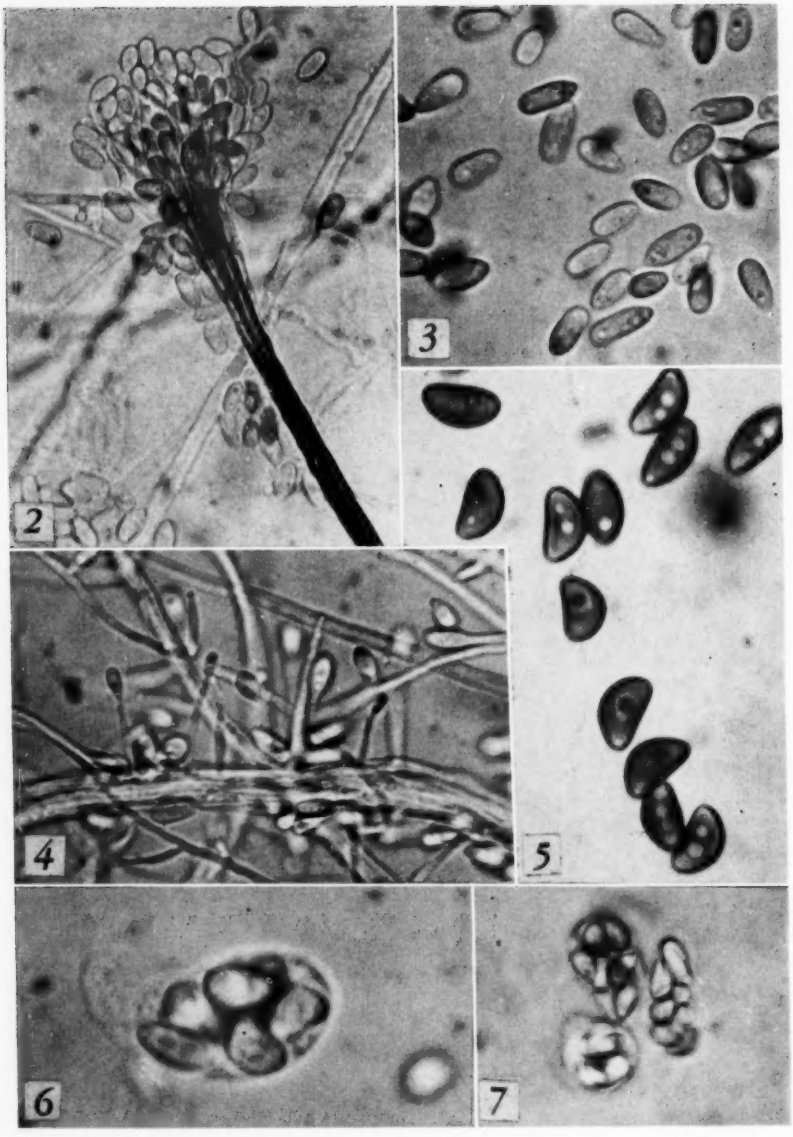



FIG. 1. (14 and 15) Perithecia of *Microascus sordidus*, (19) ascospores of *M. sordidus* (after Zukal).

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Nattrass (1937) named a fungus *Petriella asymmetrica* Curzi var. *cypria* Nattrass with the conidial state *Sporocybe cypria* Nattrass. This was isolated from the cortex of *Populus nigra*, Nicosia, Cyprus, R. M. Nattrass No. 367, October 1933. We have seen neither the publication nor the specimen.

**Petriella guttulata** Barron and Cain sp. nov. (Figs. 2–7, 13)

Peritheciis nigris, carbonaceis, subglobosis vel piriformibus, 100–250 $\mu$ diam., superficialibus, pilosis, supra collo brevi crasso papilliformi praeditis; pilis peritheciorum, firme brevibus septatis, brunneis, levibus, in parte superiore dense dispersis circiter $100 \times 1 \cdot 5$ $\mu$; ascis octosporis, ovoideis vel late clavatis, basi in stipitem brevem attenuatis, 18–30 $\times$ 12–18 $\mu$, numerosis, inaequaliter dispositis, denique evanescentibus; ascosporis multiseriatis plano-convexis vel concavo-convexis, asymmetricis, 8.5–10 $\times$ 4.5–5.5 $\mu$, non septatis, rubido-brunneis, praecipue biguttulatis, in cirrhis angustis emergentibus. Coremiis atro-brunneis tenus 1 mm altis, singulis vel aggregatis; stipitibus gracilibus filiformibus erectis 6–25 $\mu$ crassis ex hyphis fuligineis fasciculatis 2.0–2.5 $\mu$ diam. compositis, apice penicillato-expansis, capitulo globoso primum muco obvoluto, conidiis in apice pallidiore hypharum a latere et a termino insertis, recentissimis a apice generatis, facile sejunctis, piriformibus vel oblongis, apice obtusis, basi in papillam truncatam attenuatis, 5–10 $\times$ 2–4 $\mu$.

Perithecia black, carbonaceous, subglobose to pyriform, 100–250 $\mu$ in diameter, with papillate ostiole, extremely setose; hairs forming a dense mat round the perithecium, usually short, septate, pigmented, smooth, about 100 $\times$ 1.5 $\mu$; asci ovoid to broadly clavate, sometimes with a well-marked foot, 18–30 $\times$ 12–18 $\mu$, numerous, irregularly disposed, walls remaining till maturity of the ascospores, finally breaking down to free the ascospores in the perithecial cavity; ascospores plano-convex to concavo-convex in one view, ellipsoid in other view, 8.5–10 $\times$ 4.5–5.5 $\times$ 4.0–4.5 $\mu$, single-celled, red-brown in color, containing a few (usually two) large oil droplets and several smaller oil droplets, extruded at maturity as a ball at the mouth of the perithecium or as a long dark-brown cirrhus in older cultures.

Conidial stages *Sporotrichum* and *Graphium*; both conidial phases produced abundantly on P.D.A.; *Sporotrichum* stage arising first with the production of numerous conidiophores from the vegetative hyphae and from aerial strands; simple, hyaline, tapering, up to 30 $\mu$ long, bearing a number of conidia at the apical end, youngest at the tip, readily displaced, hyaline, variable in shape from pyriform to long cylindric or wedge-shaped, tapered at the basal end to a narrow truncate connective, rounded at the apical end, measuring 5–10 $\times$ 2–4 $\mu$.

*Graphium* stage soon evident; vegetative hyphae grow at right angles to the substrate and anastomose and corticate to form coremia; coremia dark-brown colored, up to 1 mm high, 6–25 $\mu$ in diameter, produced singly or in clus-

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FIGS. 2–7. *Petriella guttulata*. Fig. 2. Coremium and sporiferous head of *Graphium* stage. Fig. 3. Conidia of *Graphium* stage. Fig. 4. Conidiophores and conidia of *Sporotrichum* stage. Fig. 5. Ascospores showing large oil droplets. Fig. 6. Ascus showing well-developed foot. Fig. 7. Group of asci with wall still discernible and mature ascospores inside.

ters, consisting of hyphae 2.0–2.5 μ in diameter, branching profusely at the top to form hyaline conidia-bearing hyphae; conidia similar to the *Sporotrichum* type but more uniform in shape and released in mucus to form a large gelatinous head; head at first hyaline, becoming violaceous and finally blackish at maturity; heads frequently coalescing to form large gloeoid masses several millimeters in diameter; conidia similar in shape and size to the *Sporotrichum* type but averaging slightly larger and more consistently long-cylindric in shape with sometimes an indentation near the center to form a waist.

*Collections* were made from deer dung in moist chamber, from Ethelma Lake, Kenora Dist., Ontario, by R. F. Cain, January 9, 1958 (TRTC 33681 type). As a petri-plate contaminant, West Virginia University, Morgantown, West Virginia, by H. L. Barnett, 1957, culture No. 1024. From deer dung, Indian docks, Parry Sound Dist., Ontario, by R. F. Cain, August 19, 1955 (TRTC 31516). From moose dung, Cascade Creek Canyon, Teton Co., Wyoming, by R. F. Cain, July 5, 1955 (TRTC 33015). From dung, Toronto, Ontario, by R. F. Cain, December 1935 (TRTC 33048). From partridge dung, Bei Tamsel, Germany, by P. Vogel, developed in laboratory, Toronto, December 14, 1935 (TRTC 33049).

On P.D.A. the colonies reach a diameter of 6–8 cm in 4 weeks. Colonies are white at first, turning pale greyish with the production of the *Sporotrichum* stage and then blackish in patches with the formation of the *Graphium* coremia in clumps. Growth is irregularly zonate. Perithecia are produced abundantly, at first in the aerial hyphae, later in and on the agar, especially to the outside of the colonies in older cultures. Pure white cottony overgrowths of *Sporotrichum*-bearing hyphae are sometimes found in the center of the colonies.

A number of cultures of this species have been examined from widely different substrates and locations and the colonies are fairly consistent in growth rate and appearance.

This species shows a close resemblance to *P. sordida* in conidial and cultural characters; it differs in having an extremely hairy, papillate perithecium and distinctive ascospores.

**Petriella setifera** (Schm.) Curzi. Boll. Staz. Pat. veg. Roma, 10: 380–423. 1930. (Figs. 8, 9)

Syn.: *Microascus setifer* Schmidt. Diss. Breslau, Germany, W. C. Korn. 1912.

Perithecia membranous, spherical, pale-brown to dark-brown colored, small, 75–125 μ in diameter, with short neck, covered with scattered hairs; neck with tuft of ostiolar hairs; hairs simple, rigid, septate, up to 75 μ long, smooth, hyaline to lightly pigmented; asci ovoid to clavate, sometimes with a well-marked foot, 21–25 × 12–15 μ, evanescent; ascospores asymmetrically

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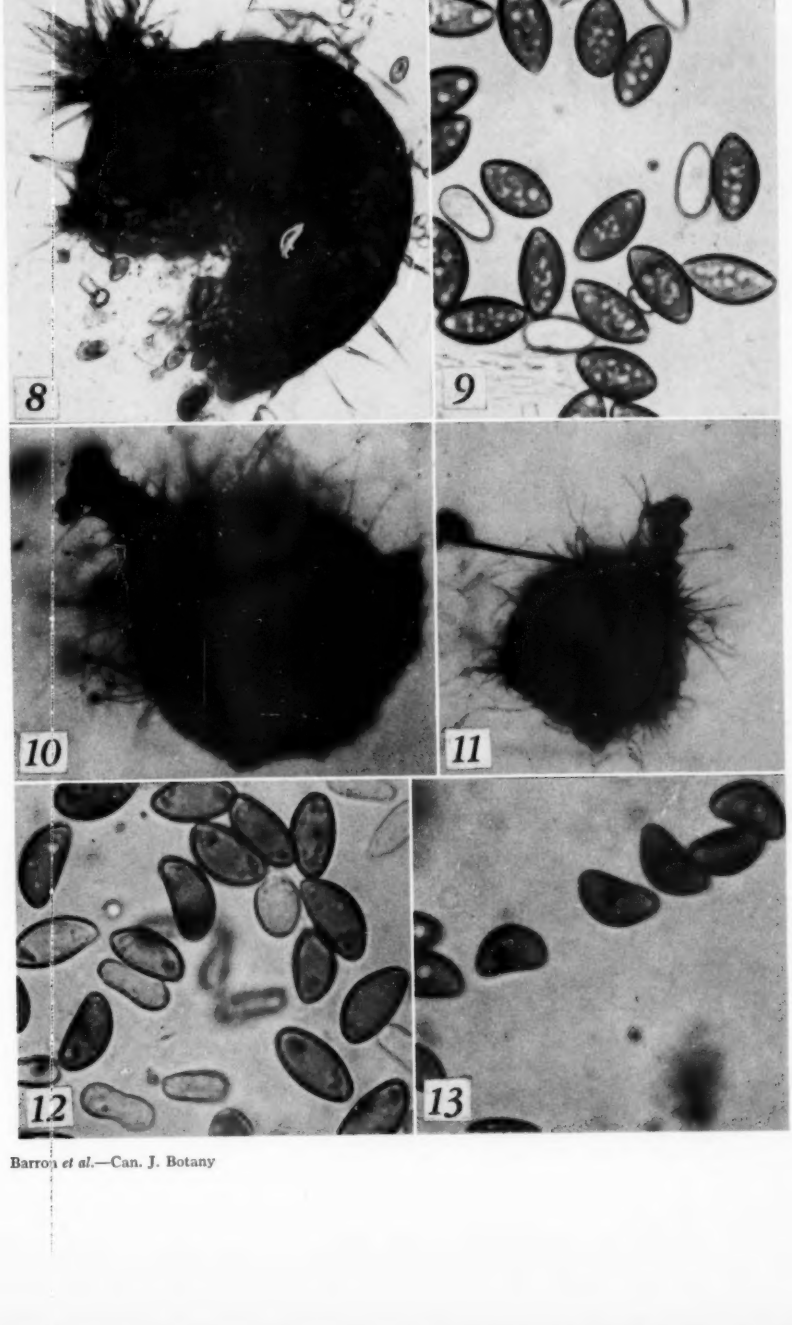
FIG. 8. Membranous perithecium of *P. setifera* with tuft of ostiolar setae.
FIG. 9. Ascospores of *P. setifera*.
FIG. 10. Perithecium of *P. sordida*.
FIG. 11. Papillate perithecium of *P. guttulata* covered with short stiff hairs. Apparent neck is remnant of the cirrhus.
FIG. 12. Ascospores of *P. sordida*.
FIG. 13. Ascospores of *P. guttulata*.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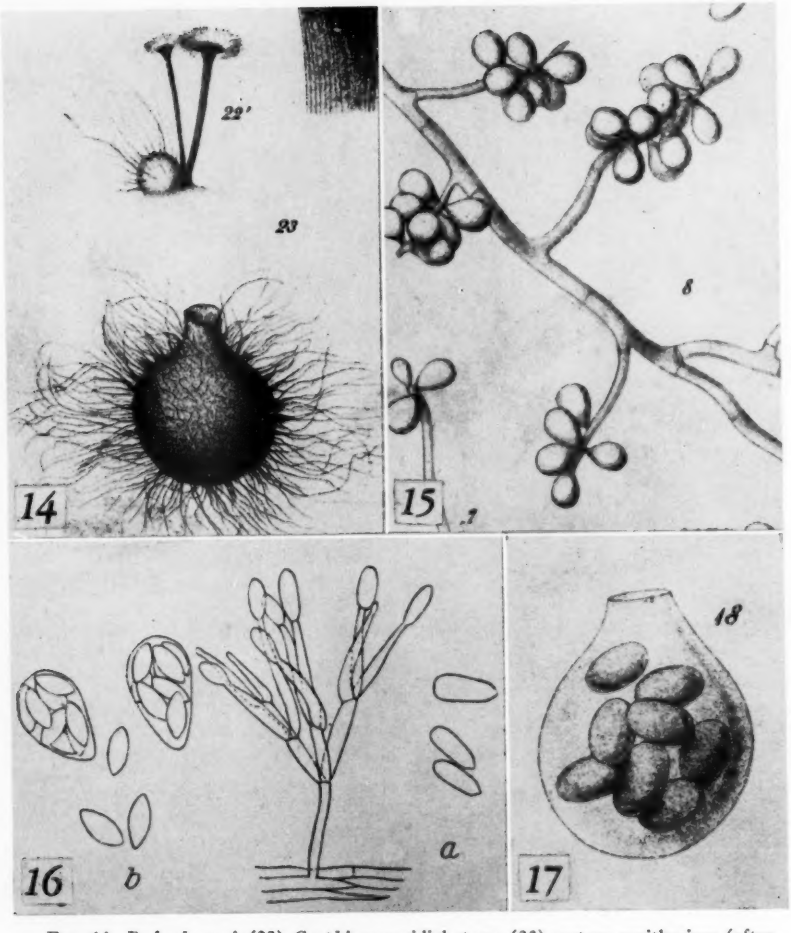


FIG. 14. *P. boulangeri*. (22) *Graphium* conidial stage, (23) mature perithecium (after Boulanger).
FIG. 15. *Sporotrichum* conidial stage of *P. boulangeri* (after Boulanger).
FIG. 16. Asci, ascospores, and conidia of *P. lindforsii* (after Lindfors).
FIG. 17. Ascus and ascospores of *P. boulangeri* (after Boulanger).

convex, pointed at the ends, sometimes faintly papillate, walls thinner at the ends to form apical germinal pores, red-brown in color, containing numerous oil droplets, measuring 7.5–11.5 μ (mostly 8.0—10.5 μ) in length by 5–6 μ in face view.

Conidial stage *Sporotrichum*; conidiophores arising from the strongly funiculose vegetative hyphae, simple, hyaline, bearing numerous conidia on short spicules near the apical end; conidia tend to unite to form loose balls; conidiophores tapering, usually between 10 and 30 μ long; conidia long cylindrical, wedge-shaped or pyriform, hyaline, tapering sharply to a narrow truncate base, 7–14 × 3–4 μ. This represents the first recording of the conidial stage of this species.

A *Graphium* conidial stage was observed in one culture soon after the study began, but it did not reappear in later work and cannot as yet be described. There seems little doubt, however, of its existence. Dr. H. L. Barnett[5] noted that it produced a synnematal stage whose occurrence was somewhat erratic and apparently dependent on substrate conditions. While all other species of *Petriella* possess a *Graphium* conidial stage, some isolates may lose the ability to produce the coremiform conidial stage after subculturing; such may be the case with the present isolate of *P. setifera*.

*Collections*: were made from horse dung, Germany, by A. Schmidt. From wilted oak tree, West Virginia, by A. L. Shigo, 1957 (924-*a*-8).

Growth of this species is very rapid on P.D.A., covering the diameter of the plate in 3 weeks at room temperature. Vegetative growth is strongly funiculose, funicles being orientated in a radial direction. *Sporotrichum* stage produced in abundance in the aerial strands and over the surface of the colonies. Colonies greyish-white at first, becoming olivaceous in the subsurface areas due to pigmentation of the vegetative hyphae. Perithecia are produced abundantly in the aerial hyphae and on the surface of the agar.

*P. setifera* is distinguished from all other species by its membranous perithecium with its tuft of ostiolar setae. Its ascospores are distinct in being asymmetrically convex with germinal pores well marked and strongly guttulate.

**Petriella lindforsii** Curzi. Boll. Staz. Pat. veg. Roma, 11: 380–423. 1931. (Fig. 16)

Perithecia blackish brown, about 350 μ broad by 250 μ high, mostly between 250 by 175 μ, solitary, papillate, covered with smooth hairs; hairs 50–100 μ long, septate, lightly pigmented, crowded round the ostiolar portion; asci oval to pyriform, 18–22 × 11–15 μ; ascospores elliptical to fusiform, not apiculate, hyaline when young, becoming red-brown at maturity, measuring 8–10 × 4.5–5.5 μ.

Conidial stage *Graphium*; coremia produced abundantly, singly or in clusters, 250–350 μ high by 20 μ broad; stalk dark-brown to black, becoming hyaline at the tip, which becomes much branched to form the conidial bearing head; conidia hyaline, cylindrical-oblong with a papillate appendage at the proximal end, 8–14 × 3.5–6 μ.

Collections: Recorded only once from the soil in Sweden.

Lindfors also described a creeping, floccose, conidial stage which he appar-

[5]Private communication.

ently regards as merely a phase of the more obvious *Graphium* stage. This phase would appear to correspond with the *Sporotrichum* stage of other closely related species. Lindfors regarded his species as being identical with that described by Boulanger (1896). Curzi, however, would regard the two species as being distinct. From a study of the available literature, Curzi seems correct in his view, and the two species appear distinct in both perithecial and ascosporic characters. The details of these differences are given under *P. boulangeri*.

The photomicrograph accompanying Lindfors' descriptions shows a perithecium very similar to that of *P. guttulata*, being papillate, somewhat pear-shaped, and covered with a dense mass of short hairs, giving a rough appearance.

**Petriella boulangeri** Curzi. Boll. Staz. Pat. veg. Roma, 11: 380–423. 1931. (Figs. 14, 15, 17)

Perithecia large, 400–500 $\mu$ in diameter, black, carbonaceous, spherical base with neck as long as the diameter of the perithecium, covered with flexuous hairs; hairs septate, pigmented dark-brown, lacking incrustations or other special characters, 100–240 $\mu$ long; asci globose, 24–30 $\times$ 18–26 $\mu$; ascospores ellipsoid, 9$\times$5 $\mu$, rounded at both ends, brownish in color, extruded at maturity in the form of a yellowish cirrhus.

Conidial stages belonging to the form genera *Sporotrichum* and *Graphium*; vegetative hyphae hyaline, bearing *Sporotrichum*-type conidiophores along their length; conidiophores simple, 30–50 $\mu$ long, tapering towards the apex, bearing as many as 12 conidia at distal end, conidia borne singly on short spicules, readily detached, hyaline, pyriform, 7–8 $\times$ 4–5 $\mu$. *Graphium* arising from *Sporotrichum*-bearing filaments after continuous culture; hyphae aggregate to form long, dark-brown coremia; hyphae in stipe of coremium branching at apex to produce hyaline filaments bearing conidia in short head; conidia long and narrow, produced in mucus, forming a transparent ball at top of dark stalk.

This species, as figured by Boulanger, may have a fairly long neck. Its ascospores are described and drawn as ellipsoid (Fig. 17). Both these characters serve to distinguish it from *P. lindforsii*, which has a papillate perithecium and fusiform ascospores (Fig. 16).

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# THE INHERITANCE AND HISTOLOGY OF A CHLOROPHYLL-DEFICIENT CHARACTER IN MEDICAGO SATIVA L.[1]

W. R. CHILDERS AND H. A. MCLENNAN

## Abstract

Further support for tetrasomic inheritance in alfalfa was shown from genetic studies of a chlorophyll-deficient seedling character. The character was determined by a single recessive gene for which the symbol Viridis-1 or $v_1$ is proposed. At the mature-plant stage, low and intermediate classes for chlorophyll production were observed. Histological examination and chlorophyll analyses confirmed this classification. The low-chlorophyll-producing class was characterized by a lack of plastids in the leaf blades although some plastids occurred in mesophyll tissue around the midrib. The intermediate chlorophyll-deficient class was characterized by yellowish-green leaf blades, associated with colorless palisade cells that overlay a single continuous layer of mesophyll cells in which the plastids were well developed.

## Introduction

Chlorophyll defects, because of their widespread occurrence in the plant kingdom and their discrete nature, have contributed much to the understanding of genetic systems. The formation of chlorophyll depends upon a suitable environment and a normal functioning of the nucleus, the cytoplasm, and the plastids. Wettstein (11) stated that, in barley, several hundred genes control the development and structural differentiation of the plastids. In the present study an apparently low production of chlorophyll was associated with normal growth and seed production, and this character was investigated to determine the quantity of chlorophyll present in the mature pale green leaves, and its mode of inheritance.

Inheritance studies are of particular interest in alfalfa because of the information they give of its origin. Alfalfa (*Medicago sativa* L.) is an established tetraploid with a low occurrence of quadrivalents at first metaphase of meiosis. In support of alfalfa's autotetraploid origin, tetrasomic inheritance has been reported by Stanford (8), and others. On the other hand, Twamley (10) and Dudley and Wilsie (4) reported both disomic and tetrasomic inheritance and Childers and McLennan (2) found evidence in support of disomic inheritance in a case of complete male sterility.

The purpose of this paper is to describe the inheritance of a chlorophyll-deficient seedling character in order to give further evidence of the autotetraploid origin of *M. sativa*. A histological study of chlorophyll-deficient leaves of mature plants was made in comparison with normal green leaves.

## Materials and Methods

An $F_1$ plant, from a cross between plants $W_3 \times$ 31-25 made for another genetic study, was found to segregate 221 normal to 7 chlorophyll-deficient

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in the $F_2$ generation. Since this frequency suggested a tetrasomic ratio (35:1), the $F_1$ plant was selfed to provide a larger segregating population.

In order to obtain $F_3$ data, 48 $F_2$ plants were grown in the greenhouse and selfed. Backcrosses were made between chlorophyll-deficient plants and heterozygous $F_2$ plants known, from $F_3$ data, to be segregating about 35:1 and 3:1.

When it was observed that chlorophyll-deficient plants became normal green in the fall under a lower light intensity, eight of these were selfed and progenies grown and classified.

Forty-five plants of the $F_2$ family 62B, segregating 3:1 for the deficient character, were selfed to produce segregating families in the $F_4$ generation. This study was made to provide further proof for a disomic or tetrasomic interpretation.

Two classes of the chlorophyll-deficient character were observed when deficient seedlings were grown to maturity. In one (class A) the leaf blades were white and the petioles and veins were green; in the other (class B) the condition was similar except that the leaf blades were pale yellowish green. A histological study of class A, class B, and normal plants was undertaken to determine the distribution of plastids in the leaf tissues. Leaves were prefixed in Carnoy's solution and placed in a modification of Karpechenko's fixative. The material was embedded in paraffin, cut at 8 $\mu$, and stained in Johansen's (5) quadruple stain.

Quantitative measurements were made of the chlorophyll content of class A and class B deficients and of normal plants. Nine leaves, excluding the petioles, were taken from each of three plants, weighed, then ground in 85% acetone made slightly alkaline with $Na_2CO_3$. The resulting slurry was filtered and the residue washed with 85% and then 100% acetone. The filtrate was made up to volume with 85% acetone.

The optical density of each filtrate was determined by means of a Bausch and Lomb Spectronic 20 Photoelectric colorimeter at a wavelength of 6630 Å. An optical density versus chlorophyll concentration curve had been worked out previously for this instrument using a Beckman model DU spectrophotometer and the specific absorption coefficients of MacKinney (6), after the method of Arnon (1). The results were expressed as milligrams of total chlorophyll (chlorophyll *a* plus chlorophyll *b*) per gram fresh weight of leaf tissue.

## Results

The results of this study are divided into two parts; one concerned with the mode of inheritance of the chlorophyll-deficient character and the other with leaf histology and related studies.

### Inheritance Studies

$F_1$ plants from the cross $W_3 \times$ 31-25 were green, showing that the chlorophyll-deficient character was recessive. $F_2$ segregation data for normal:chlorophyll-deficient, together with tests for goodness of fit for alternate tetrasomic (35:1 random chromosome segregation and 19.25:1 chromatid segregation) and disomic (63:1 trihybrid and 15:1 dihybrid) ratios were as follows:

| Green obs. | Chl. deficient obs. | $\chi^2$ | $P<$ | Ratio fitted |
|---|---|---|---|---|
| 947 | 29 | 0.18 | .70 | 35:1 |
| | | 7.98 | .01 | 19.2:1 |
| | | 12.45 | .01 | 63:1 |
| | | 17.80 | .01 | 15:1 |

Only the 35:1 tetrasomic ratio gave a good fit. The actual ratio 32.6:1 lies within the limits of 19.25 and 35:1, which would be expected if some double reduction due to chromatid segregation had occurred.

The distribution of variates in 33 $F_3$ progenies from $W_3 \times$ 31-25 was observed (Table I) in order to test the validity of the hypothesis based on $F_2$ data. Three segregation classes occurred. On a tetrasomic interpretation, seven out of eight families showed a good fit to a 3:1 ratio (simplex), and 12 out of 15 families to a 35:1 ratio (duplex). The remaining 10 families did

TABLE I

Test for goodness of fit to assumed ratios in 33 $F_3$ families from the cross $W_3 \times$ 31-25

| | Observed | | Tetrasomic | | | Disomic | | |
|---|---|---|---|---|---|---|---|---|
| $F_2$ parents | Normal | Chlor. def. | $\chi^2$ | $P>$ | Ratio fitted | $\chi^2$ | $P>$ | Ratio fitted |
| 62B | 378 | 118 | .38 | .50 | 3:1 | .38 | .50 | 3:1 |
| 170 | 269 | 87 | .05 | .50 | 3:1 | .05 | .50 | 3:1 |
| 53 | 19 | 9 | .76 | .30 | 3:1 | .76 | .30 | 3:1 |
| 51 | 541 | 119 | 8.99* | .00 | 3:1 | 8.99* | .00 | 3:1 |
| 173 | 95 | 26 | 1.90 | .20 | 3:1 | 1.90 | .20 | 3:1 |
| 45 | 160 | 39 | 3.08 | .05 | 3:1 | 3.08 | .05 | 3:1 |
| 49 | 206 | 57 | 1.53 | .20 | 3:1 | 1.53 | .20 | 3:1 |
| 62A | 198 | 55 | 1.43 | .20 | 3:1 | 1.43 | .20 | 3:1 |
| 175 | 134 | 6 | 1.16 | .20 | 35:1 | .89 | .30 | 15:1 |
| 172 | 220 | 1 | 4.32* | .02 | 35:1 | 12.68* | .00 | 15:1 |
| 171 | 195 | 5 | .05 | .50 | 35:1 | 4.8* | .02 | 15:1 |
| 82 | 570 | 21 | 1.33 | .20 | 35:1 | 7.4* | .00 | 15:1 |
| 79 | 227 | 6 | .04 | .50 | 35:1 | 5.3* | .01 | 15:1 |
| 64 | 180 | 3 | .89 | .30 | 35:1 | 6.6* | .01 | 15:1 |
| 61 | 1376 | 24 | 5.76* | .01 | 35:1 | 49.1* | .00 | 15:1 |
| 56 | 107 | 5 | 1.49 | .20 | 35:1 | .60 | .30 | 15:1 |
| 54 | 266 | 2 | 4.01* | .02 | 35:1 | 13.2* | .00 | 15:1 |
| 52 | 32 | 1 | .01 | .50 | 35:1 | .61 | .30 | 15:1 |
| 44A | 120 | 2 | .60 | .30 | 35:1 | 4.28* | .01 | 15:1 |
| 43A | 215 | 7 | .01 | .50 | 35:1 | 3.57 | .05 | 15:1 |
| 18 | 89 | 1 | .84 | .30 | 35:1 | 4.03* | .02 | 15:1 |
| 14 | 66 | 1 | .40 | .50 | 35:1 | 2.60 | .10 | 15:1 |
| 48 | 37 | 1 | .01 | .50 | 35:1 | .85 | .30 | 15:1 |
| 15 | 435 | 0 | | | | | | |
| 43B | 76 | 0 | | | | | | |
| 46 | 313 | 0 | | | | | | |
| 60 | 968 | 0 | | | | | | |
| 167 | 120 | 0 | | | | | | |
| 176 | 189 | 0 | | | | | | |
| 177 | 158 | 0 | | | | | | |
| 178 | 49 | 0 | | | | | | |
| 17 | 397 | 3 | | | | | | |
| 55 | 854 | 1 | | | | | | |

*A significant deviation from ratios fitted.

not segregate or segregated in a ratio similar to 575:1 (triplex). When a disomic interpretation was considered, 7 out of 8 families showed a good fit to a 3:1 ratio (monohybrid), and 6 out of 15 families to a 15:1 ratio (dihybrid).

The genotypic frequency expected in an $F_2$ progeny segregating 35:1 is 9 non-segregating families, 18 segregating 35:1, 8 segregating 3:1, and 1 homozygous recessive. The ratio expected for a disomic segregation is 7:4:4:1. The distribution of the first three classes for both tetrasomic and disomic segregation showed (Table II) that the observed ratio fitted the expected tetrasomic ratio but did not fit the expected disomic ratio.

TABLE II

Observed and expected tetrasomic and disomic ratios for the normal: chlorophyll-deficient character in the $F_3$ families of the cross $W_3 \times$ 31-25

| | Non-* segregating | Segregating (35:1 tetrasomic 15:1 disomic) | Segregating (3:1 tetrasomic and 3:1 disomic) | $\chi^2$ | $P>$ |
|---|---|---|---|---|---|
| Observed | 10 | 15 | 8 | | |
| Expected tetrasomic (9:18:8) | 8.5 | 17 | 7.5 | .53 | .70 |
| Disomic (7:4:4) | 15.4 | 8.8 | 8.8 | 6.32 | .02 |

*This class includes both quadruplex and triplex families.

Backcrosses between chlorophyll-deficient plants and $F_2$ plants segregating 35:1 and 3:1 were made. The segregation of these backcross progenies showed that all families fitted the expected ratios (Table III).

TABLE III

Test for goodness of fit to assumed ratios in eight families of $F_2$ heterozygous plants backcrossed to recessive chlorophyll-deficient plants

| Families | Observed | | $\chi^2$ | $P>$ | Ratio fitted |
|---|---|---|---|---|---|
| | Norm. green | Chl. def. | | | |
| Segregating 35:1 | | | | | |
| 1 | 68 | 15 | .14 | .70 | 5:1 |
| 2 | 17 | 5 | .54 | .30 | 5:1 |
| Segregating 3:1 | | | | | |
| 3 | 10 | 12 | .18 | .50 | 1:1 |
| 4 | 6 | 7 | .08 | .70 | 1:1 |
| 5 | 26 | 16 | 2.40 | .10 | 1:1 |
| 6 | 14 | 14 | .00 | .99 | 1:1 |
| 7 | 4 | 5 | .12 | .70 | 1:1 |
| 8 | 4 | 11 | 3.20 | .05 | 1:1 |

$F_4$ families were classified for the chlorophyll-deficient character and both 3:1 and 35:1 ratios occurred in the frequency of 11 and 8 respectively. These data support the tetrasomic interpretation.

Eight chlorophyll-deficient plants that had become green in the fall were selfed. Their progenies were all chlorophyll-deficient with the exception of

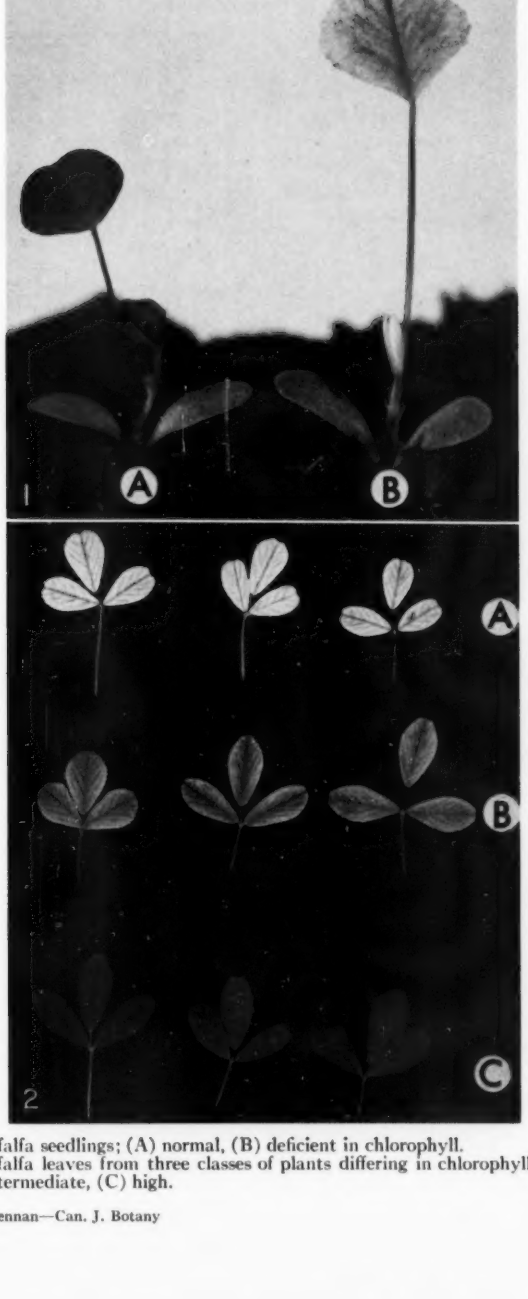
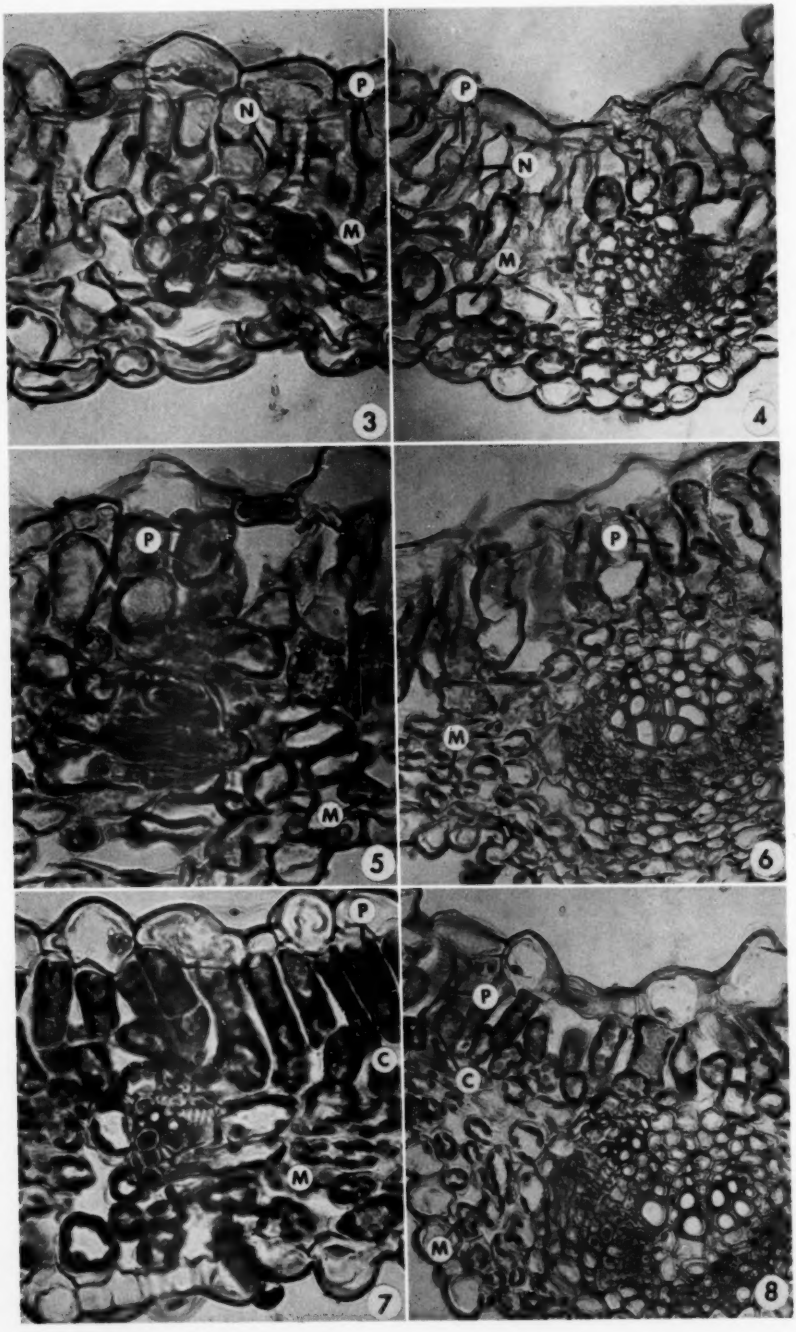



FIG. 1. Alfalfa seedlings; (A) normal, (B) deficient in chlorophyll.
FIG. 2. Alfalfa leaves from three classes of plants differing in chlorophyll production; (A) low, (B) intermediate, (C) high.

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one seedling in each of two families. The number of selfed progeny in these families varied from 7 to 71.

## Histological and Related Studies

The chlorophyll-deficient character was first observed in the seedling stage (Fig. 1). In the $F_2$ seedlings the cotyledons were green, but in the $F_3$ seedlings the cotyledons were, in many cases, pale green. In either case the unifoliate leaf was slightly flecked with green whereas the true leaves that followed had white or yellow green leaf blades and green veins and midribs.

Two classes of chlorophyll-deficient leaves, one low in chlorophyll production and the other intermediate, were observed when recessive seedlings were grown to maturity in the greenhouse. Low-chlorophyll-producing leaves were shown by plants Cl 26-1, Cl 3-5, and 62A-11 (Fig. 2A), intermediate chlorophyll-producing leaves were shown by plants 49-17, 49-19, 49-6 (Fig. 2B), and normal high-chlorophyll-producing leaves were shown by three clonal plants of the $F_1$ parent (Fig. 2C). In late fall all the chlorophyll-deficient plants turned green and remained green until late winter when, under intense light, the new shoots were again chlorophyll deficient. Most plants grew to maturity and produced seeds.

The results of the chlorophyll analysis (Table IV) showed that the low-chlorophyll-producing plants (class A) had 19.2% of the chlorophyll content of normal plants. Class B deficients were intermediate in this respect and had 43.0% of the normal plants.

TABLE IV

Chlorophyll analysis of nine leaves per plant in clones differing in chlorophyll production

| Chlorophyll production | Chlorophyll concentration, mg/g fresh wt. | Mean |
|---|---|---|
| Class A | | |
| 1 | 0.502 | |
| 2 | 0.532 | 0.511 |
| 3 | 0.498 | |
| Class B | | |
| 1 | 1.131 | |
| 2 | 1.143 | 1.142 |
| 3 | 1.153 | |
| Normal | | |
| 1 | 2.619 | |
| 2 | 2.688 | 2.655 |
| 3 | 2.659 | |

Histological studies showed that leaves of low-chlorophyll-producing plants had broader and shorter palisade cells (Fig. 3) than normal leaves (Fig. 7).

Figs. 3 to 8. Cross sections of alfalfa leaves. Figs. 3 and 4. Low-chlorophyll class; vein and midrib sectors respectively. Figs. 5 and 6. Intermediate-chlorophyll class; vein and midrib sectors respectively. Figs. 7 and 8. High-chlorophyll class; vein and midrib sectors respectively. Figs. 3, 5, and 7, ×600. Figs. 4, 6, and 8, ×360. C, chloroplasts; M, mesophyll cell; N, nucleus; P, palisade cell.

Chloroplasts were not observed and the nuclei were prominent in both palisade and mesophyll tissues. The mesophyll cells surrounding the main midrib contained chloroplasts (Fig. 4). The main vascular bundle and the leaf blades were considerably less developed than in the normal leaf (Fig. 8).

In the intermediate-chlorophyll-producing plants, the palisade cells were similar to those of the low-chlorophyll-producing type (Fig. 5). Chloroplasts were not observed in the palisade cells but, unlike those of the low-chlorophyll type, were present in cells around the small vascular bundles and in a single continuous layer of mesophyll cells of the leaf blade. Chloroplasts were also observed in both mesophyll and palisade cells surrounding the midrib (Fig. 6).

## Discussion

Although the character was discrete in the seedling stage, it was not possible to distinguish between the low- and intermediate-chlorophyll-producing classes. These classes were not apparent until the plants were more mature and therefore did not enter into the genetic analysis. The appearance of an intermediate class in the segregating generations, most particularly in the $F_3$ family 49 (Table I) could be attributed to other genetic factors. This intermediate-chlorophyll-producing type is under further genetic study. The genetic ratios support a one-gene interpretation, eliminating the possibility of a dosage effect.

Genetic data compiled from seedling classification indicated that the chlorophyll-deficient character was determined by a single recessive gene inherited tetrasomically. To conform with the classification of chlorophyll-deficient mutations of Wettstein (11), the proposed symbol for this gene is *viridis*-1 or $v_1$. The gene apparently causes an arrested development of the chloroplasts.

The *viridis* character described here was different from the mottled, chlorophyll-deficient gene reported by Stanford and Cleveland (9). Their mottled gene was also inherited tetrasomically but the character was reported to have a high mutation rate.

Chlorophyll-deficient plants became green under the low-light intensity of the fall and early winter seasons. Darlington (3) observed in *Vicia faba* a similar reversion to green under low-light intensity. He noted that differences in temperature did not effect the progress of change from chlorophyll-deficient to green. This study as well as the present one indicated that no permanent damage was done to the proplastids in the affected palisade and mesophyll tissues.

Histological observations showed that the intermediate class was characterized by both a continuous, single-celled green layer of mesophyll tissue underlying the colorless palisade layer in the leaf blades and a greater concentration of normal mesophyll and palisade cells surrounding the main vascular bundles. Rhoades (7) observed a similar gene-controlled condition, normal mesophyll tissue underlying colorless palisade tissue, which produced yellow stripes in corn leaves. Chlorophyll analysis in the present study showed that the intermediate class was also intermediate in the production of chlorophyll.

The advantage of large populations was demonstrated in this study. In the $F_2$, where the families were large, disomic and tetrasomic ratios were readily distinguishable by a test of the goodness of fit. In some $F_3$ families

where the populations were small, good fits were obtained for both disomic and tetrasomic ratios. In many genetic studies with alfalfa that have been made since tetrasomic inheritance was reported by Stanford (8), populations have often been too small to differentiate between the two types of inheritance.

## Acknowledgments

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# TRANSLOCATION OF $C^{14}$ IN TOBACCO AT DIFFERENT STAGES OF DEVELOPMENT FOLLOWING ASSIMILATION OF $C^{14}O_2$ BY A SINGLE LEAF[1]

MICHI SHIROYA, G. R. LISTER, C. D. NELSON, AND G. KROTKOV

## Abstract

Single leaves of tobacco plants at four different stages of development were allowed to carry on photosynthesis in $C^{14}O_2$ for 30 to 50 minutes followed by photosynthesis in air. The total elapsed time from the beginning of assimilation was 5½ hours. Redistribution of $C^{14}$ was determined in: I, a young plant with eight leaves; II, an older plant with 19 leaves; III, a mature flowering plant with 29 leaves; IV, a mature seed plant with 35 leaves. The leaf treated with $C^{14}O_2$ in each of the four plants was number seven, counting from the bottom. This leaf was only half expanded in the youngest plant but fully expanded in the other three plants.

The treated leaf of the youngest plant translocated 22% of the total $C^{14}$ fixed, twice as much as the treated leaves of the other three plants. The large amount of translocation in the youngest plant occurred from a leaf that contained 28% of its ethanol-soluble fraction as sugar phosphates and only 25% as sucrose. The treated leaves of the older plants that translocated half as much radioactivity had less $C^{14}$ in sugar phosphates and more $C^{14}$ in sucrose. For example, the treated leaf of the seed plant contained 13% of its soluble $C^{14}$ in sugar phosphates and 70% in sucrose.

Sucrose and some hexose was recovered from the stems and roots of all plants. Amino acids were recovered from both stem and root of the youngest plant but not from these parts of the three older plants. Since 80 to 100% of the $C^{14}$ in the stems was recovered as sucrose it is concluded that this sugar is the main form in which carbon is translocated in tobacco. Although sugar phosphates and organic acids were found in the treated leaves of all plants they were not detected in stems or roots and were apparently not translocated.

All plants translocated $C^{14}$ to leaves both below and above the treated leaf. Two factors influenced the destination of $C^{14}$. One was the anatomical connection among leaves, and the second was the stage of development of any particular leaf. Although $C^{14}$ was translocated to leaves at nodes bearing flowers no $C^{14}$ was translocated to these flowers or to developing or mature seeds in any of these plants.

## Introduction

When one leaf of a plant is allowed to carry on photosynthesis in $C^{14}O_2$, radioactive carbon may be distributed to all parts of the plant. However, the pattern of distribution is not uniform but is affected by many different conditions.

One factor affecting the pattern of distribution is the position of the assimilating leaf. Belikov (3) has shown with soya bean that low-level leaves contribute $C^{14}$-labelled assimilates mainly to the roots, but as the plant matures, ever-increasing numbers of leaves pass their assimilates to the developing fruit. Also, it has been shown that assimilation products are not translocated between mature leaves but move from them into young growing leaves. Jones *et al.* (6) have demonstrated similar distribution patterns among leaves

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of tobacco. In addition they found labelled assimilates only in leaves above the one fed $C^{14}O_2$ and not at all in the lower leaves even though there was translocation to the root. Distribution patterns among leaves also depend on the presence of vascular connections. Leaves that have no connection with the assimilating leaf are not supplied (6).

The metabolic activity of the organ receiving assimilates has a great effect on the distribution patterns. For example, absorption of ions by roots of soya bean (14) can increase the flow of assimilates to this organ, while chilling roots drastically inhibits the flow of assimilates in both sugar cane (4) and soya bean (11). Developing fruits receive large amounts of assimilates from all parts of the plant. On the basis that the movement of phosphate from leaves is correlated with the movement of the products of photosynthesis, $P^{32}$-distribution patterns are of interest in this connection. In *Pisum sativum*, Link (9) has found that phosphate moves most rapidly from a leaf into a pod attached at the same axil. Both $P^{32}$ and $C^{14}$ have been used to show how the developing seeds of sunflower receive assimilates (15, 16), each leaf of the plant apparently supplying a specific file of seeds in the developing head.

Conditions affecting the metabolic activity of the supply leaf may affect entry into the conducting stream and thus alter distribution patterns. In the leaf of *Rheum*, amino acids and organic acids enter conducting tissues at different rates (8). It is also interesting to note that enriching sugar beet leaves with adenosine triphosphate accelerated the release of assimilates to the conducting tissue (7).

The present work with tobacco was carried out to show the amount and nature of the $C^{14}$-labelled assimilates translocated and the contribution of a single leaf to the various organs at four different stages of development of the plant. In addition the amounts of $C^{14}$ in the ethanol-insoluble fraction and among the various compounds of the ethanol-soluble fraction of the plant tissues were measured.

## Materials and Methods

*Nicotiana tabacum*, var. Connecticut Havana 38, was grown from seed planted May 1 and kept under greenhouse conditions. Plants were grown in soil in 12-inch pots and given a weekly feeding with a commercial fertilizer, Plant Prod 20-20-20. With a day temperature of 27–30° C and a night temperature of 20–23° C and with medium to long days the plants started to produce flowers after 3 months and had finished producing seed after 4½ months.

Four plants, 2 months old, were chosen and the seventh leaf of each, counting from the bottom, was marked. This leaf, called the treated leaf, was allowed to carry on photosynthesis in $C^{14}O_2$ when plants had reached various stages of development as shown in Fig. 1. Plant I, with eight leaves, had none that were more than half expanded. Plant II, with 19 leaves, had four leaves fully expanded, the treated leaf and the three leaves above. Plant III, with 29 leaves, had eight fully expanded leaves and inflorescences of flowers with developing fruit. Plant IV, with 35 leaves, had 10 fully expanded leaves and inflorescences of dried seed pods containing seeds.

An assimilation experiment using $C^{14}O_2$ was carried out in the greenhouse

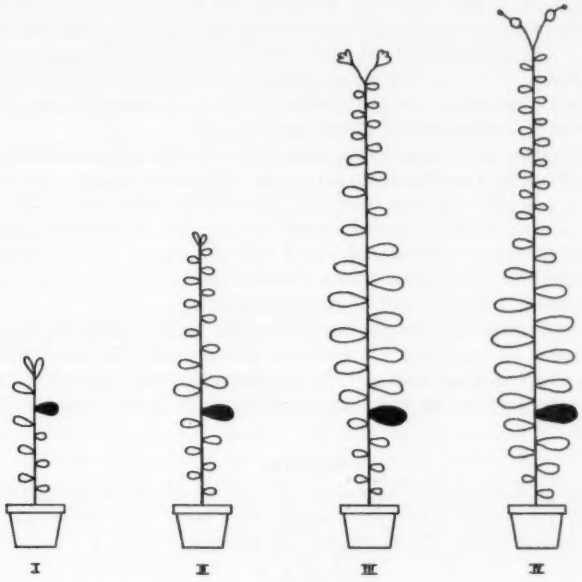


FIG. 1. Diagrams of the tobacco plants used in experiments at the four different stages of development.
I. Young plant, 68 days old, eight leaves.
II. Mature plant, 81 days old, 19 leaves.
III. Flowering plant, 107 days old, 29 leaves.
IV. Seed plant, 135 days old, 35 leaves.

with each of the four plants as follows. The marked leaf (Fig. 1) was sealed with silicone grease into a transparent chamber, 0.76 l. in volume for the leaf of plant I and 8.1 l. for the leaves of the other plants. The chamber was connected as previously described (12) to a closed circuit of tubing into which was introduced a pump, a reservoir, and a Geiger tube connected to a ratemeter. In this closed system 280 $\mu$c of $C^{14}O_2$, specific activity 35 mc per mM and an initial concentration of carbon dioxide in air of 0.036%, was circulated over the leaf for 30 to 50 minutes, that is until compensation point was reached as shown by the ratemeter. The leaf then was removed from the chamber and allowed to carry on photosynthesis in air for an additional period so that the total elapsed time from the beginning of assimilation was 5½ hours. Illumination was daylight and varied between 1500 and 2000 ft-c measured at the surface of the treated leaf as previously described (10). The temperature varied between 20 and 25° C.

At the end of each experiment all leaves were detached from the stems and weighed and their outlines traced on paper for subsequent determination of area. Roots were separated from the soil by careful washing in water. All parts, leaves, stem, and root, were extracted three times with hot 80%

ethanol. Aliquots of the combined washings for each plant part were reduced to a convenient volume and plated on aluminum planchets for determination of soluble $C^{14}$. Insoluble residues were converted to barium carbonate by the method of Baker *et al.* (2) for determination of insoluble $C^{14}$. All radioactive measurements were made using a methane flow proportional counter with an efficiency of 50% as previously described (12).

The distribution of $C^{14}$ among the compounds of the ethanol-soluble fraction was obtained using two-dimensional paper chromatography and autoradiography. The solvent systems used for chromatography were 80% aqueous phenol containing 0.03% concentrated ammonium hydroxide followed by double development in *n*-propanol, ethyl acetate, water, 7:1:2. Radioactivity was determined by plating aliquots of solutions of the various compounds eluted with water from the paper chromatograms.

Results were calculated on the basis that the total $C^{14}$ recovered from each plant was equal to the total $C^{14}$ fixed by the treated leaf even though it is known that there is some loss of $C^{14}O_2$ by leaves in the light (10). All results reported are subject to an error not greater than $\pm$ 8% based on standard deviations.

## Results

A measure of the total $C^{14}$ translocated can be obtained by comparing the radioactivity recovered from the treated leaf with that recovered from all other parts of the plant. These results are shown in Table I.

TABLE I

Total $C^{14}$ fixed and translocated by one leaf of tobacco at four different stages of development

| | | Plant I | Plant II | Plant III | Plant IV |
|---|---|---|---|---|---|
| Area of treated leaf | $cm^2$ | 218 | 625 | 500 | 830 |
| Total $C^{14}$ fixed (recovered) | $\mu c$ | 169.1 | 173.1 | 105.9 | 154.3 |
| Total $C^{14}$ left in treated leaf | % | 78 | 90 | 89 | 89 |
| Total $C^{14}$ translocated | % | 22 | 10 | 11 | 11 |

In the youngest plant (I) 22% of the total $C^{14}$ was translocated out of the treated leaf in 5½ hours. In plant II, 10%, and in III and IV 11%, of the $C^{14}$ was translocated. Therefore, twice as much $C^{14}$ was translocated in the young plant from a leaf less than half expanded than in the three older plants.

The destination of the translocated $C^{14}$ is shown in Table II. In plant I, the bulk of the translocated $C^{14}$ was concentrated in the ethanol-soluble, 31.5 $\mu c$, and -insoluble, 2.9 $\mu c$, fractions of the stem. A small amount of soluble $C^{14}$ was recovered from the root, 1.7 $\mu c$, and the other leaves, 0.6 $\mu c$. While some $C^{14}$ was recovered in the insoluble fraction of the leaves no $C^{14}$ was detected in this fraction in the root. However, the large bulk of the root made detection of insoluble activity difficult. In plant II, the main destination of $C^{14}$ was the stem with a very small amount recovered from the root. In this plant the leaves received more $C^{14}$, 3.2 plus 3.0 $\mu c$, than the root. In the flowering and seed plants very little $C^{14}$ was translocated to the leaves while considerable $C^{14}$ was recovered in the stems and roots. It is interesting to

TABLE II

Distribution (in $\mu c$) of $C^{14}$ in the ethanol-soluble and -insoluble fractions of various parts of tobacco at four different stages of development*

| | Plant I | | Plant II | | Plant III | | Plant IV | |
|---|---|---|---|---|---|---|---|---|
| | Sol. | Insol. | Sol. | Insol. | Sol. | Insol. | Sol. | Insol. |
| Treated leaf | 72.3 | 58.9 | 103.3 | 52.6 | 66.3 | 27.0 | 64.9 | 71.8 |
| Stem | 31.5 | 2.9 | 6.8 | 3.3 | 7.6 | 3.2 | 7.8 | 4.9 |
| Root | 1.7 | 0† | 0.9 | 0† | 1.8 | 0† | 5.9 | 0† |
| Other leaves | 0.6 | 0.7 | 3.2 | 3.0 | Trace | Trace | Trace | Trace |
| Flowers and fruit | — | — | — | — | 0 | 0 | — | — |
| Seeds and pods | — | — | — | — | — | — | 0 | 0 |
| Total $C^{14}$ recovered | 106.1 | 62.5 | 114.2 | 58.9 | 75.7 | 30.2 | 77.6 | 76.7 |

*Zero means less than 10 mμc unless otherwise indicated. Trace means 10 to 50 mμc.
†Zero means less than 100 mμc. The limit is higher here because of the difficulty of determining small amounts of $C^{14}$ in the presence of large amounts of cold carbon.

note that in the treated leaf of plant IV, the $C^{14}$ content of the insoluble fraction exceeded that of the soluble fraction, while in all other plants the amount of soluble $C^{14}$ in the treated leaf was up to two times greater than the amount of insoluble $C^{14}$. In spite of this high amount of soluble $C^{14}$ in the treated leaves of plants II, III, and IV, translocation from treated leaves of these plants was only half as great as that from the treated leaf of plant I.

Distribution of $C^{14}$ among the compounds of the soluble fraction of the treated leaves is shown in Fig. 2. With advancing age the amount of sugar

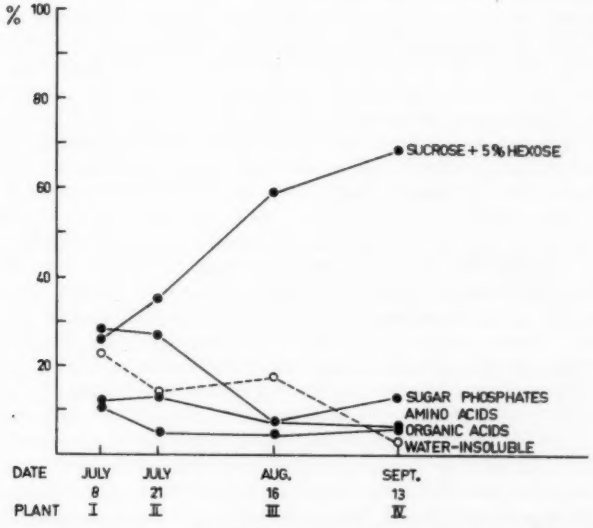


FIG. 2. Distribution of radioactivity among the compounds of the ethanol-soluble fractions of the treated leaves of tobacco at different stages of development.

increased from 25% of the soluble fraction in the youngest plant to 70% of the soluble fraction in the seed plant. At the same time the sugar phosphates decreased from 28% to 13% and the ethanol-soluble–water-insoluble fraction (lipids, pigments) decreased from 22% to 2%. The organic acid and amino acid fractions remained relatively unchanged. It is surprising that the leaves of the older plants contained such high amounts of sucrose and yet translocated so little of this reservoir of sugar, when compared to the younger plants.

Distribution of $C^{14}$ among the soluble compounds of the stem and root was also determined. The results are shown in Table III. In all plants the

TABLE III

Distribution (in %) of $C^{14}$ among the ethanol-soluble compounds of the stem and root of plants at different stages of development

| | Plant I | | Plant II | | Plant III | | Plant IV | |
|---|---|---|---|---|---|---|---|---|
| | Stem | Root | Stem | Root | Stem | Root | Stem | Root |
| Sucrose | 90 | 90 | 80 | 100 | 100 | 99 | 100 | 100 |
| Hexose | 5 | 5 | 20 | 0 | 0 | 1 | 0 | 0 |
| Amino acids | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sugar phosphate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Organic acids | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

bulk of the soluble $C^{14}$ in both stem and roots was in sucrose. In fact, in the seed plant sucrose was the only compound detected in both stem and root. This evidence supports the idea that sucrose is the main form in which carbon is translocated in tobacco. Only the youngest plants contained radioactive amino acids in the stem and root. Since the distribution in stem and root was the same, these amino acids were probably translocated from the leaf and did not arise from metabolism of translocated sucrose. Although sugar phosphates accounted for a large proportion of the soluble $C^{14}$ in the treated leaf, these compounds were not detected in the stem or root of any of the plants. Even plant I, which produced more sugar phosphates than sugar, apparently did not translocate sugars in the phosphorylated form. Organic acids were also present in the treated leaves but were not detected in the stem or root of any of the plants and, therefore, were not translocated.

The leaves of the tobacco plant can be identified in order from the treated leaf, those above being 1, 2, 3. . . , and those below $-1$, $-2$, $-3$. . . . The phyllotaxis can be determined as described by Allard (1) and was 3/8, clockwise, in the tobacco plants used, the most frequent phyllotaxis found in many varieties of tobacco (1). When the distribution of $C^{14}$ in the various leaves of each plant was determined it was found to correspond very closely to the 3/8 phyllotaxis. Figure 3 and Table IV (column six) illustrate this in plant II. Leaves 4 and 12 of this plant were opposite the treated leaf, that is they were at an angular distance of 4/8 and contained no $C^{14}$. Leaves 1, 7, and 9 with an angular distance from the treated leaf of 3/8 contained 7.2, 8.5, and 5.0 mμc respectively; leaves 2 and 6 with an angular distance of 2/8 contained 10 and 10.4 mμc; leaves 3, 5, and 11 with a distance of 1/8 contained 18.5, 79.1, and 207 mμc; while leaf 8 directly above the treated

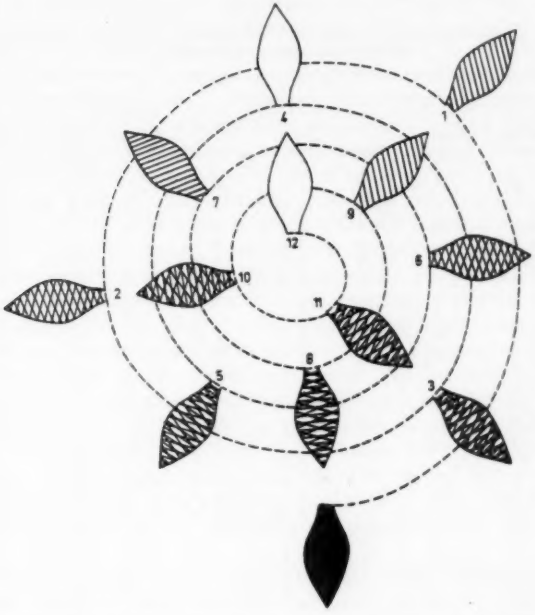


FIG. 3. Phyllotaxis and distribution of $C^{14}$ in plant II, 81 days old. The treated leaf is shown in black. The degree of cross-hatching indicates the relative amounts of ethanol-soluble $C^{14}$ recovered.

leaf contained 1475 mμc. Only leaf 10 was an exception to this carefully regulated pattern. If this pattern, as shown in Fig. 3, is compared to the results of dye-injection experiments in apple, described by Roach (17), it is evident that one conducting strand from the treated leaf supplied all the other leaves of the plant.

The distribution patterns recorded in Table IV show that in plants I and II all the leaves below the treated leaf received $C^{14}$. This is in contradiction to the results obtained by Jones *et al.* (6) for tobacco. In plants III and IV less $C^{14}$ was recovered from the leaves below the treated leaf and many leaves received no $C^{14}$.

The distribution of $C^{14}$ in the leaves above leaf 13 of plant III generally correlated with angular distance from the treated leaf. This was also true for the leaves of plant IV that contained $C^{14}$. However, leaves 1 to 13 of plant III and leaf 16 of plant IV contained no $C^{14}$. Many of these leaves were in the same orthostichy as the treated leaf and on this basis should have been radioactive. When distribution of $C^{14}$ was correlated with leaf area it was evident that $C^{14}$ was not imported by older, fully expanded leaves. With the exception of leaves 1 to 4, those leaves close to the treated leaf on plants II and IV, this generalization holds for all the plants. It is concluded

TABLE IV

Comparison of phyllotaxis and leaf area with distribution of $C^{14}$ in the leaves of tobacco at different stages of development*

| Leaf | Distance from treated leaf | Plant I | | Plant II | | Plant III | | Plant IV | |
|---|---|---|---|---|---|---|---|---|---|
| | | Area ($dm^2$) | $C^{14}$ ($m\mu c$) | Area ($dm^2$) | $C^{14}$ ($m\mu c$) | Area ($dm^2$) | $C^{14}$ ($m\mu c$) | Area ($dm^2$) | $C^{14}$ ($m\mu c$) |
| −6 | 2/8 | 1.4 | 7.3 | 1.5 | 10.5 | — | — | 1.8 | 1.5 |
| −5 | 1/8 | 1.6 | 27.3 | 2.0 | 2.7 | 2.1 | 0 | 2.4 | 0 |
| −4 | 4/8 | 1.8 | 2.7 | 3.1 | 3.0 | 2.1 | 0 | 4.1 | 0 |
| −3 | 1/8 | 2.0 | 17.6 | 3.1 | 9.9 | 2.4 | 1.8 | 6.1 | 0 |
| −2 | 2/8 | 2.2 | 20.5 | 4.0 | 9.0 | 2.9 | 2.8 | 7.2 | 0 |
| −1 | 3/8 | 2.5 | 6.0 | 4.4 | 3.4 | 3.0 | 0 | 7.2 | 0 |
| Treated leaf | | 2.2 | | 6.3 | | 5.0 | | 8.3 | |
| 1 | 3/8 | 1.4 | 10.5 | 5.9 | 7.2 | 5.9 | 0 | 8.5 | 7.6 |
| 2 | 2/8 | | | 7.0 | 10.0 | 7.2 | 0 | 8.5 | 7.5 |
| 3 | 1/8 | | | 6.2 | 18.5 | 7.3 | 0 | 9.3 | 8.6 |
| 4 | 4/8 | | | 5.4 | 0 | 7.0 | 0 | 7.1 | 5.2 |
| 5 | 1/8 | | | 4.9 | 79.1 | 7.0 | 0 | 8.0 | 0 |
| 6 | 2/8 | | | 3.7 | 10.4 | 6.7 | 0 | 8.0 | 0 |
| 7 | 3/8 | | | 2.5 | 8.5 | 6.8 | 0 | 7.1 | 0 |
| 8 | 0 | | | 2.0 | 1475.0 | 7.0 | 0 | 5.9 | 3.2 |
| 9 | 3/8 | | | 1.3 | 5.0 | 6.5 | 0 | 5.7 | 0 |
| 10 | 2/8 | | | 0.7 | 1004.0 | 5.3 | 0 | 4.0 | 0 |
| 11 | 1/8 | | | 0.2 | 207.0 | 4.9 | 0 | 4.0 | 2.8 |
| 12 | 4/8 | | | — | 0 | 4.7 | 0 | 2.0 | 0 |
| 13 | 1/8 | | | | | 4.2 | 0 | 2.0 | 0 |
| 14 | 2/8 | | | | | 3.3 | 5.0 | 1.2 | 0 |
| 15 | 3/8 | | | | | 2.1 | 0 | 2.7 | 0 |
| 16 | 0 | | | | | 1.1 | 2.3 | 1.5 | 0 |
| 17 | 3/8 | | | | | 1.3 | 2.7 | 0.8 | 0 |
| 18 | 2/8 | | | | | — | 5.0 | | 8.3 |
| 19 | 1/8 | | | | | 0.4 | 1.0 | | 0 |
| 20 | 4/8 | | | | | 0.5 | 0 | | 0 |
| 21 | 1/8 | | | | | 0.9 | 1.8 | | 0 |
| 22 | 2/8 | | | | | 1.0 | 1.0 | | 0 |
| 23 | 3/8 | | | | | | | | 0 |
| 24 | 0 | | | | | | | | 0 |
| 25 | 3/8 | | | | | | | | 0 |
| 26 | 2/8 | | | | | | | | 0 |
| 27 | 1/8 | | | | | | | | 0 |
| 28 | 4/8 | | | | | | | | |

*Zero means less than 1 $m\mu c$.

that two factors influenced the destination of $C^{14}$, one was the anatomical connection between leaves and the second was the stage of development of the receiving leaf.

Table IV also shows that in plant III carbon was received by leaf 22, the farthest removed from the treated leaf. However, developing fruit borne on axillary inflorescences from leaf 18 to 22 did not contain $C^{14}$ (Table II). Also, in spite of the fact that many of the young developing leaves on plant IV contained $C^{14}$ the fully developed seeds of this plant were not radioactive.

## Discussion

These experiments with tobacco plants of different ages were set up to approach as closely as possible the conditions under which the plants were

grown. The administration of $C^{14}O_2$ was carried out under the normal conditions of temperature and illumination occurring in the greenhouse. In addition, $C^{14}O_2$ was fed at or close to the concentration existing in air. By using $C^{14}$ of high specific activity it was possible to keep the period of assimilation short and still introduce enough radioactivity into these large plants to allow careful analysis. The total time of 5½ hours for distribution of label from the treated leaf was chosen after consideration of experiments with soya bean and tobacco. Clauss *et al.* (5) found that primary leaves of soya bean kept in the light translocated the bulk of the photosynthetically assimilated $C^{14}$ that was able to be translocated in the first 2 hours after the fixation period had ended and very little after 6 hours. Immediately following a period in the dark there was some additional translocation. Jones *et al.* (6) found that in tobacco the supply to leaves above the fed leaf was essentially complete after 4 to 5 hours and restricted to the period 6 to 7 hours after assimilation.

The most surprising observation in the present experiments is that no $C^{14}$ was translocated to the developing seeds. All previous work with $P^{32}$ and $C^{14}$ (3, 9, 15, 16) showed that developing fruits are the main destination of assimilated carbon. In fact as the pod develops in soya beans even the lowest leaves on the plant pass their assimilates to the fruit (3). On the other hand, in tobacco, the young leaves, far above the treated leaf and at the same nodes as the developing flowers, were radioactive. This indicates that the failure to translocate to developing seeds was not due to the lack of a vascular connection.

In soya bean (5) and tobacco (6) large pools of both soluble and insoluble carbohydrate remained in the fed leaf and were not translocated. Jones *et al.* (6) showed that this irreversibly incorporated $C^{14}$ may account for as much as 60% of the total $C^{14}$ fixed in older leaves of tobacco but only 40% in very young leaves. In addition, activity in sugar formed a higher proportion in the ethanol-soluble fraction in the oldest as compared with the youngest leaf. They used leaves of different ages from plants that were at a stage of development comparable to plant II in the present studies. The same observations have now been made using leaves of different ages from plants at different stages of development including flowering and seed plants. Thus, it is concluded that the differences in translocation from young leaves is really a function of age of the leaf and not a function of its relation to the ontogeny of the plant. Also, the ability of young leaves to translocate more $C^{14}$ than old leaves may be correlated with their ability to phosphorylate sugar or to the level of adenosine triphosphate present in conducting tissue as Kursanov (7) and Pavlinova (13) suggest. The leaves that were the best suppliers of assimilates in tobacco were those that contained a very high proportion of sugar phosphates. In the older leaves some factor for the transfer of sucrose from mesophyll tissue to conducting tissue may be limiting. This would explain the lack of translocation of sucrose from the leaf when this sugar appears to be the form in which carbon is translocated in the stem.

In all four plants tested there was translocation of considerable amounts of $C^{14}$ to leaves below the treated leaf. However, less $C^{14}$ was translocated to lower leaves in the older plants until, in the seed plant, only one of the lower

leaves was radioactive. This effect of age may explain why Jones *et al.* (6) failed to find radioactivity in leaves below the treated leaf. It may not be necessary, therefore, to explain their results, as Kursanov has done (7), by a transfer of material from phloem to xylem.

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# THE EFFECT OF NUTRITION ON SYNNEMATA FORMATION IN HIRSUTELLA GIGANTEA PETCH[1]

T. C. LOUGHHEED


## Abstract

The influence of nutrition on synnemata formation in *Hirsutella gigantea* has been studied. A number of carbon sources were satisfactory but a "crude glucose" preparation was the best. Only two nitrogen sources gave good yields of synnemata and both were rather complex mixtures. Two other compounds, phosphoglyceric acid and gibberellic acid, both known stimulants of other morphological processes, also increased synnemata production.

An apparent light requirement of synnemata formation may also be satisfied by some compound(s) in "crude glucose", liver fraction L, and autolyzed yeast. Phosphoglyceric acid and gibberellic acid were more effective stimulants in the dark than in the light.

Synnemata were formed earlier and were more plentiful if the medium was inoculated by flooding rather than with a loop.

A medium which will support the growth of *H. gigantea* and the production of synnemata within 4 weeks has been formulated.

It is concluded that the diversity of factors influencing synnemata formation indicates that the morphological differentiation is a multistep process which may be affected at several points.


## Introduction

The repeated isolation of a fungus, tentatively identified by Mains (11) as *Hirsutella gigantea*, from naturally infected spruce budworm larvae (*Choristoneura fumiferana* (Clem.)) suggested that the fungus might be of some value in the biological control of this insect. *H. gigantea* grew readily on artificial media but, unfortunately, was sterile. As spores are the ideal form in which to disseminate a fungus in the field, a study of the factors influencing spore formation was undertaken.

The influence of numerous factors, both physical and chemical, on spore formation has been studied extensively and has recently been summarized by Hawker (4). *H. gigantea* is a member of the family Stilbaceae, which is characterized by the fascicular, spore-bearing structures called coremia or synnemata (Figs. 1 and 2). The early literature concerning coremia and synnemata formation has been reviewed by Hejmánková-Uhrová and Hejmánek (8) but very little appears to be known about the physiology of their development. Hejmánek and Hejmánková-Uhrová in a series of papers on coremia formation in *Trichophyton mentagrophytes* Kaufmann-Wolf (5, 6, 7, 8) have postulated that desiccation of the culture, a pH change in the medium, and an accumulation of metabolic products are all important factors in stimulating formation of coremia. While these factors did occur in their cultures the data presented do not appear to be conclusive in proving a causal relationship. The concentration of glucose in the media affected coremia formation, 10% being optimal. No other nutritional studies were reported. When four different subcultures of the original *T. mentagrophytes* isolate were

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grown in mixed cultures with each other, with other *Trichophyton* species, and with some other fungi, a marked stimulation of coremia formation was obtained. Growing two colonies of the same subculture in one dish was not effective.

More recently Taber (13) and Taber and Vining (14) have investigated synnemata production in *Isaria cretacea* van Beyma. Synnemata developed over a narrower temperature range than general vegetative growth; the initiation of the synnemata seemed to be the sensitive stage. A low pH in the medium also discouraged synnemata formation. They found that the nutritional requirements were not too critical in regard to either the nature or concentration of the nutrients. Two vitamins, biotin and thiamine, were essential and synnemata formation required higher concentrations than did vegetative growth. A wide variety of carbon and nitrogen sources were satisfactory although fewer carbon sources permitted good synnemata production than permitted general colony growth. Sucrose and mannitol, while favoring abundant mycelial production, completely suppressed synnemata development when present in the medium even at low concentrations. The inhibitory action could be partially reversed by the addition of buffers or indoleacetic acid. The latter compound is itself an inhibitor of mycelial growth. Furthermore, Taber has shown that mycelium of *I. cretacea*, if washed free of exogenous nutrients and incubated in a moist chamber on filter paper, can utilize the endogenous nutrients to form synnemata.

Since synnemata appear to be a necessary prerequisite to spore formation in *H. gigantea*, a study of the factors influencing their development has been undertaken. Some effects of nutrition on the formation of synnemata are reported in this paper.

## Materials and Methods

The organism used in these experiments was isolated by MacLeod (9) from naturally infected spruce budworm larvae and was maintained by routine transfers on Sabouraud maltose agar.

The dispersed mycelium, used as inoculum, was prepared as described by MacLeod (10) except that the saline solution was replaced by a citrate–phosphate buffer (0.1 *M*, pH 6). It was found that dispersed mycelium, prepared in this way, was viable for periods up to 1 month if stored in a refrigerator.

All the experiments described here were carried out on nutrient agar. Preliminary work had shown that extended incubation periods were likely to be involved and that petri plates dried out too soon to be satisfactory. Accordingly, 8-ounce, flat-sided bottles were used as culture vessels. As a general procedure, the required nutrients, melted agar, and water were dispensed into the bottles to give a total volume of 25 ml. The bottles were plugged with non-absorbent cotton and autoclaved at 15 pounds pressure for 15 minutes. After autoclaving, the bottles were cooled, on their sides, as quickly as possible to minimize degradation of the medium due to heat. When cool, the media were inoculated with a loopful of dispersed mycelium placed centrally on the surface of the agar.

The cultures were incubated at 24° C in the dark. After a short period in

PLATE I

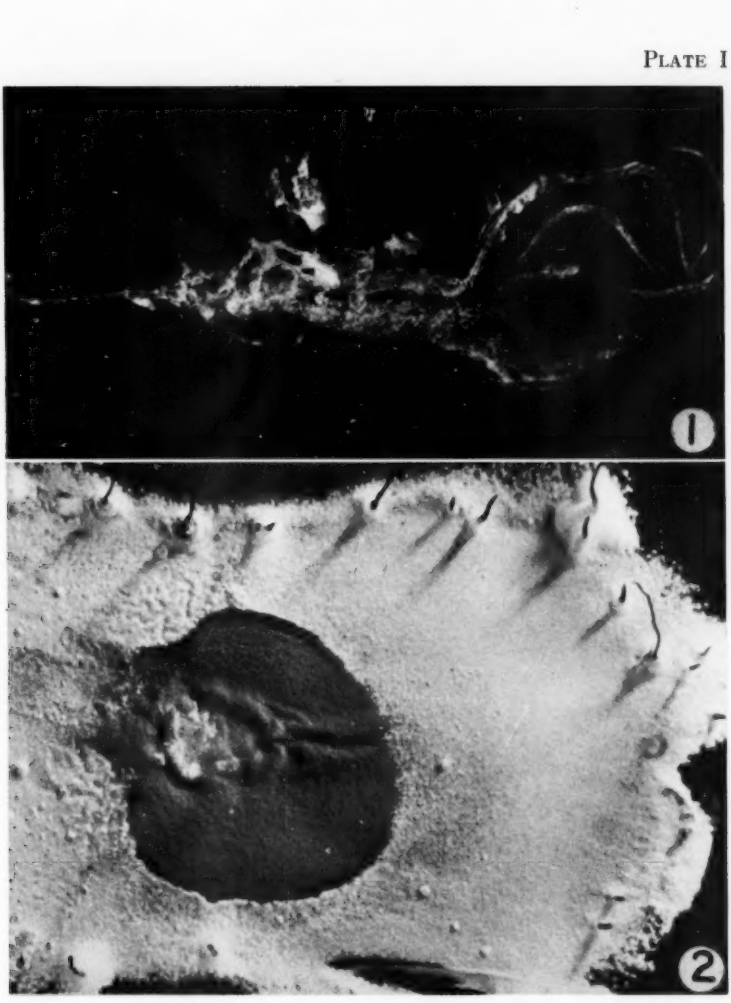

FIG. 1. Synnemata of *H. gigantea* growing from a naturally infected spruce budworm larva ×7.
FIG. 2. Synnemata of *H. gigantea* growing on an artificial medium ×2.

a horizontal position, to allow the excess water in the inoculum to be absorbed, the bottles were transferred to the more convenient upright position. At the end of 8 to 12 weeks, depending mainly on the rate of development of the fungus on the media being tested, the cultures were exposed to light from a "Cool White" fluorescent lamp (75 foot-candles) for 16 hours. The cultures were subsequently returned to the dark and observed further.

The process of synnemata production can be considered as consisting of two phases: an initiation step, followed by an elongation due to growth. Three sets of data were collected in order to show the effectiveness of the nutrients for each phase and also for the over-all effect, as follows:

(1) The total length of synnemata; to measure the over-all effectiveness of the treatment.

(2) The number of synnemata; to measure the initiation phase.

(3) The mean length of the synnemata; to measure the growth phase.

For each of the replicate cultures, (1) and (2) were measured and from these (3) was calculated. The results shown in the tables are the averages of the values in each set of data so obtained, together with their standard errors. Since the number of replicates varied between experiments, the numbers used are given with each experiment. The sources of the chemicals and other commercial preparations used are also given in the appropriate Experimental section. The "crude glucose" was obtained from Dr. A. G. Morton, Akers Research Laboratories, Imperial Chemical Industries Ltd., Welwyn, Great Britain.

## Experimental

*Effect of Carbon Source*

Sixteen carbon sources, selected mainly for their ability to support the growth of *H. gigantea* in submerged culture, were tested for their effect on synnemata formation. The results are shown in Table I.

TABLE I

The effect of carbon sources on synnemata formation

| Carbon source | No. of replicates | Synnemata | | |
|---|---|---|---|---|
| | | Av. total length per culture (mm) | Av. no. per culture | Av. mean length per culture (mm) |
| Crude glucose | 3 | 202.0±39.2 | 26.0± 4.0 | 8.2±2.1 |
| Glucose (Kerfoot)* | 2 | 191.0±37.0 | 14.0± 3.0 | 13.7±5.0 |
| Mannose (B.D.H.) | 2 | 144.0±26.0 | 32.0±10.0 | 4.7±0.7 |
| Cellobiose (Light) | 2 | 98.0±18.0 | 14.0± 4.0 | 7.2±1.1 |
| Glycogen (B.D.H.) | 2 | 98.0±68.0 | 16.5± 6.5 | 5.1±2.3 |
| Fructose (B.D.H.) | 3 | 70.3±22.6 | 8.7± 0.8 | 8.5±2.8 |
| Maltose (Kerfoot) | 3 | 31.0±18.2 | 9.7± 4.1 | 2.9±0.6 |
| Sorbitol (B.D.H.) | 3 | 25.7±10.8 | 14.7± 7.2 | 1.9±0.4 |
| Starch (H. and W.) | 3 | 15.3± 7.5 | 6.3± 2.9 | 2.5±0.3 |
| Mannitol (B.D.H.) | 3 | 14.3± 8.1 | 3.7± 1.3 | 3.7±1.2 |
| Sucrose (B.D.H.) | 3 | 7.7± 2.4 | 2.0± 0.6 | 4.3±1.5 |
| Trehalose (Pfan.) | 2 | 2.5± 1.3 | 2.5± 1.3 | 1.0±0.0 |

* Kerfoot, Thomas Kerfoot and Co. Ltd.; B.D.H., British Drug Houses Ltd.; Light, L. Light and Co. Ltd.; H. and W., Hopkin and Williamson; Pfan., Pfanstiehl Laboratories, Inc.

NOTE: Basal medium contained peptone (0.5%) and agar (2%). Incubated in darkness at 24° for 16 weeks, illuminated, incubated further 4 weeks. Number of replicates as indicated.

The crude glucose medium was the only one on which synnemata formed before the light exposure. Therefore, since it also produced the greatest overall yield and the second greatest number of synnemata, it must be considered the best carbon source. The largest number of initiation sites occurred on the mannose medium, while the glucose medium was best in respect to growth. There was no sharp demarcation in the results obtained with the various carbon sources, but only a gradual decrease in the number and size of synnemata. Glycerol, ribose, lactose, and stearic acid media supported very little growth of the organism and no synnemata formed on any of these cultures.

Later experiments showed that equally good results were obtained when crude glucose and glucose were used at a concentration of 1%. This lower level was, therefore, used in subsequent work. In addition, media used for later experiments were prepared containing 1.5% agar. This variation caused no apparent difference in the results obtained.

*Nitrogen Source*

Eight nitrogen sources ranging in complexity from a single amino acid to complex protein mixtures were tested for their effect on synnemata formation. Since some earlier experiments had shown that the nitrogen concentration might be important, the compounds were tested at three concentrations (0.16, 0.08, and 0.02% nitrogen). The results are given in Table II.

It is evident that two compounds, liver fraction L and autolyzed yeast, gave results far superior to those of any other compound tested. The earlier observations on the importance of nitrogen level were fully confirmed. Both initiation and growth were optimal at the same level for each of the compounds. In addition, synnemata were formed on both media in the dark, as in the case of crude glucose.

TABLE II

The effect of different nitrogen sources on synnemata formation

| | | Synnemata | | |
|---|---|---|---|---|
| Nitrogen source | Concn. (%) | Av. total length per culture (mm) | Av. no. per culture | Av. mean length per culture (mm) |
| Liver fraction L (NBCo.)* | 1.34 | 46.3±11.8 | 36.7±10.2 | 1.3±0.1 |
| | 0.67 | 185.7±41.4 | 75.7± 3.0 | 2.4±0.5 |
| | 0.17 | 0.7± 0.7 | 1.7± 1.7 | 0.1±0.1 |
| Autolyzed yeast (Difco) | 1.95 | 37.3± 9.5 | 17.0± 2.5 | 2.2±0.4 |
| | 0.98 | 120.7±29.7 | 36.7± 5.1 | 3.2±0.4 |
| | 0.24 | Nil | — | — |
| Neopeptone (Difco) | 1.11 | 2.0± 1.0 | 2.0± 1.0 | 0.7±0.5 |
| | 0.56 | 9.0± 4.7 | 6.0± 3.0 | 1.0±0.5 |
| | 0.14 | 6.3± 2.6 | 3.3± 1.9 | 2.2±0.4 |
| Tryptone (Difco) | 1.22 | Nil | — | — |
| | 0.61 | Nil | — | — |
| | 0.15 | 5.7± 0.3 | 5.7± 0.3 | 1.0±0.0 |
| Yeast extract (Difco) | 1.95 | Nil | — | — |
| | 0.98 | 1.3± 0.7 | 1.0± 0.6 | 1.0±0.6 |
| | 0.24 | 3.7± 2.2 | 5.3± 3.5 | 0.8±0.1 |

* NBCo., Nutritional Biochemicals Corporation; Difco, Difco Laboratories Incorporated.
NOTE: Basal medium contained glucose (1%) and agar (1.5%). Incubated in darkness at 24° for 13 weeks, illuminated, incubated further 4 weeks. Three replicate cultures used.

Three of the media tested failed to support any synnema formation. These were casein hydrolyzate (acid), glutamic acid – trace elements, and glutamic acid – trace elements – yeast extract. The trace elements and yeast extract supplements were added to the chemically pure glutamic acid so that it compared more closely with the other crude compounds tested.

*Phosphoglyceric Acid*

Hawker (3) first showed that the sugar phosphates, glucose 1-phosphate and fructose diphosphate, stimulated sporulation of the fungus *Melanospora destruens* Shear. Buston *et al.* (1) obtained similar results with *Chaetomium globosum* Kunze and, later, Buston and Khan (2) showed that phosphoglyceric acid (PGA) was an even more effective stimulant of perithecia formation in *C. globosum*.

In preliminary experiments with these three compounds it was found that only PGA had an effect on synnemata formation by *H. gigantea*. It also appeared that this compound was effective only when the concomitant nutritional conditions were optimal. Accordingly, the effect of PGA was investigated in more detail.

The results, given in Table III, indicate that PGA is an effective stimulant of both synnemata initiation and growth in *H. gigantea*. Maximum stimulation of both was obtained in cultures grown in the dark, though at different levels of PGA. The addition of PGA to the glucose–neopeptone medium, previously found to be poor for obtaining synnemata, increased its effectiveness to a level comparable to the glucose – liver fraction L and glucose – autolyzed yeast media.

TABLE III

The effect of phosphoglyceric acid on synnemata formation

| | | Synnemata | | |
|---|---|---|---|---|
| | Phosphoglyceric acid concentration (%) | Av. total length per culture (mm) | Av. no. per culture | Av. mean length per culture (mm) |
| Light | 0.1 | 50.8± 7.2 | 23.3±4.7 | 2.3±0.3 |
| | 0.02 | 34.5± 6.4 | 16.8±2.9 | 2.1±0.1 |
| | Nil | 1.3± 1.3 | 0.8±0.8 | 0.4±0.4 |
| Dark | 0.1 | 121.3±33.8 | 51.8±8.1 | 2.2±0.3 |
| | 0.02 | 47.0±13.1 | 15.3±3.1 | 3.0±0.1 |
| | Nil | 1.8± 0.8 | 1.8±0.6 | 0.8±0.3 |

NOTE: Basal medium contained glucose (1%), neopeptone (0.5%), and agar (1.5%). Incubated at 24° for 13 weeks with one set in light and other in dark. Four replicate cultures used.

*Gibberellic Acid*

Gibberellic acid is now well known as a stimulator of growth and flowering in a large number of plants and, since the *Hirsutella* synnema is a stalk-like growth, it was thought possible that gibberellic acid might be effective in promoting the growth phase of synnema formation.

The procedure was identical with that used for PGA except that gibberellic acid (Nutritional Biochemicals Corporation, 10% K salt) was added at three levels; 0.01%, 0.001%, and 0.0001%. The results are shown in Table IV.

TABLE IV

The effect of gibberellic acid on synnemata formation

| | Gibberellic acid concentration (%) | Synnemata | | |
|---|---|---|---|---|
| | | Av. total length per culture (mm) | Av. no. per culture | Av. mean length per culture (mm) |
| Light | 0.01 | 21.3± 2.9 | 8.5±2.0 | 2.9±0.7 |
| | 0.001 | 25.5± 9.9 | 12.0±3.6 | 2.0±0.2 |
| | 0.0001 | Nil | — | — |
| | Nil | Nil | — | — |
| Dark | 0.01 | 56.8±14.4 | 12.0±3.7 | 5.6±1.0 |
| | 0.001 | 99.5±19.5 | 28.5±3.1 | 3.7±0.8 |
| | 0.0001 | Nil | — | — |
| | Nil | 0.3± 0.3 | 0.3±0.3 | 0.3±0.3 |

NOTE: Basal medium contained glucose (1%), neopeptone (0.5%), and agar (1.5%). Incubated at 24° for 13 weeks with one set in light and one in darkness. Four replicate cultures used.

It is evident that some stimulation of growth was obtained, most notably in those cultures grown in the dark. Unexpectedly, gibberellic acid was also a good stimulant of initiation, although somewhat poorer than PGA.

The cultures, which had been grown in the dark, were exposed to the standard light treatment and many of the white synnemata, especially the smaller ones, changed to a dark green-black color. In some of the cultures many dark spots also appeared in the mycelial mat, presumably due to the formation of a similar pigment. This would suggest that these spots, usually numbering between 100 and 150, are initiation sites where differentiation of the mycelium has started but where synnema formation has not yet progressed to the point where they are visible structures.

*Formulation of Media for Synnemata Formation*

Based on the results reported above, a number of media were formulated to determine which gave maximum synnemata production. Only those compounds which supported synnemata formation in the dark were used.

*Carbon source.*—Since synnemata formed in the dark, and in the greatest over-all yield, on crude glucose medium, this compound was used as the carbon source in all the media at a concentration of 1%.

*Nitrogen source.*—Two preparations, liver fraction L and autolyzed yeast, were almost equally satisfactory. Each was used, separately, at its optimum concentration of 0.67% and 0.98% respectively.

*Stimulatory compounds.*—Since PGA gave somewhat better results than gibberellic acid, it was used in these experiments at a level of 0.1%. To determine if its stimulatory powers were supplementary to those of the carbon and nitrogen sources, PGA was included in only half the cultures of each set.

*Inoculation.*—In all previous work the cultures were inoculated with a loopful of dispersed mycelium placed centrally on the agar. However, it was noted that small isolated colonies, probably due to splashing of the inoculum when the loop was removed, tended to have a disproportionately large number of synnemata. Timnick *et al.* (15) have shown that flooding of the agar, as

TABLE V

The effect of various nutrients and inoculation techniques on synnemata formation

| Nitrogen source | PGA | Inoculation | Synnemata | | | |
|---|---|---|---|---|---|---|
| | | | Av. total length per culture (mm) | Av. no. per culture | Av. mean length per culture (mm) | Av. time to appearance (weeks) |
| Liver fraction L | PGA | Loop | 55.0±50.6 | 14.0± 9.8 | 1.9±1.4 | 12–16 |
| Liver fraction L | — | Loop | 32.0±15.5 | 9.0± 4.0 | 2.8±0.9 | 12–16 |
| Liver fraction L | PGA | Flooding | 144.7±31.4 | 64.0± 2.9 | 2.3±0.1 | 4 |
| Liver fraction L | — | Flooding | 134.3±24.6 | 65.3± 3.0 | 2.1±0.2 | 4 |
| Autolyzed yeast | PGA | Loop | 12.0±12.0 | 10.0±10.0 | 0.4±0.4 | 12–16 |
| Autolyzed yeast | — | Loop | 17.0±15.2 | 4.0± 3.0 | 2.3±1.3 | 12–16 |
| Autolyzed yeast | PGA | Flooding | 200.3±22.2 | 54.0± 8.1 | 3.8±0.6 | 8 |
| Autolyzed yeast | — | Flooding | 189.3±23.7 | 53.7± 5.9 | 3.5±0.1 | 8 |

NOTE: Basal medium contained crude glucose (1%) and agar (1.5%). Incubated in darkness at 24° with periodic examination for 16 weeks. Three replicate cultures used.

compared with inoculation at one point, greatly decreased the time required for spore formation in *Melanconium fuligineum* (Scrib. and Viala) Cav. and several other fungi. Accordingly, the two methods of inoculation were tried with these media. To flood the bottles, 0.5 ml of dispersed mycelium was pipetted onto the agar surface and spread by tilting the bottles. These cultures, then, received more inoculum, but each growing point had less dispersed mycelium than those cultures inoculated in the ordinary way.

Table V summarizes the composition of the media and the results obtained.

The most striking result was the superiority of flooding as the method of inoculation, due mainly to a large increase in the number of initiation sites. In the presence of the complex carbon and nitrogen sources the addition of PGA was without effect. Apparently the nature of the stimulatory effect detected previously is similar to that provided by the nitrogen and (or) carbon sources. The two nitrogen sources tested gave essentially similar results, except in one respect. With liver fraction L synnemata were formed in a shorter time than with autolyzed yeast.

## Discussion

An examination of the figures given in this paper will show that there is a considerable variation in the results reported. It will also be seen that, in general, the greater the over-all yield, the less is the variability. However, the scatter is such that fine distinctions cannot be made, only the large differences may be relied upon.

It appears that synnema formation is controlled to a large extent by nutritional conditions and there may be a dual requirement since, in some cases, both the chemical nature and the concentration must be correct. The carbon source is not too critical but a "crude glucose" preparation was the most satisfactory. Morton *et al.* have shown (12) that this "crude glucose" contains a sporulating factor for *Penicillium griseofulvum* Dierckx but it is not known if this factor is active here. The nitrogen requirement is much more specific and only a few complex nitrogen sources within a limited concentration range were effective. In view of the stimulation obtained with phosphoglyceric acid and gibberellic acid, it is not certain whether the effectiveness of these nitrogen sources is due to their nitrogenous constituents or to some other compound(s) in these complex mixtures.

Preliminary work had shown the effectiveness of light in stimulating synnemata formation. However, as the work proceeded it became evident that light is not essential since "crude glucose", liver fraction L, autolyzed yeast, PGA, and gibberellic acid were all effective in the dark. It appears, therefore, that one requirement of the fungus for synnemata formation may be met either by exposure to light or by supplying some chemical compound.

The efficacy of inoculation by flooding, as compared to the usual method, is not readily explained. The multiplicity of growing points may exhaust the nutrient sooner, as suggested by Timnick *et al.* (15), but the consequences of this action are uncertain. It is possible that this rapid utilization of nutrients leads to an accumulation of intra- or extra-cellular metabolites which favors synnemata formation, with the additional possibility that a shunt mechanism may be operative. Alternatively, the rapid growth may exhaust an inhibitor

so quickly that synnemata formation may then proceed while other conditions are still favorable.

The diversity of the factors influencing synnemata formation would seem to indicate that it is a multistep process which may be affected at several points. Therefore a detected requirement might be for any one of these steps. Furthermore, Taber (13) has recently reported that *Isaria cretacea* can form synnemata utilizing only endogenous nutrients. It is not yet clear if this is a general situation but, until more is known of the actual processes of synnema formation, results dealing with nutritional requirements must be interpreted with caution.

## Acknowledgments

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# LEAF AND SHOOT BLIGHT OF POPLARS (SECTION TACAMAHACA SPACH) CAUSED BY VENTURIA POPULINA (VUILL.) FABRIC.[1]

B. W. DANCE[2]

## Abstract

This study is concerned with the recognition, pathology, and taxonomy of *Venturia populina* (Vuill.) Fabric. (=*Didymosphaeria populina* Vuill.), and its imperfect stage *Pollaccia elegans* Serv., which is shown to cause a leaf and twig blight of balsam poplar (*Populus balsamifera* L.) in North America. *Venturia populina* may be confined to hosts of the section *Tacamahaca* Spach of the genus *Populus*, although experimental evidence presented here suggests that poplars of the section *Aigeiros* Duby also may be susceptible, as they are in Europe. The binomial *Fusicladium radiosum* (Lib.) Lind, which was listed by Servazzi as a *pro parte* synonym of *P. elegans*, is shown to have been commonly applied in North America to the fungus representing *Pollaccia radiosa* (Lib.) Bald. & Cif., a pathogen which is confined to poplars of the section *Leuce* Duby in both Europe and North America. *Fusicladium radiosum* (Lib.) Lind var. *balsamiferae* J. J. Davis, however, is shown to be a synonym of *P. elegans*.

## Introduction

Relatively recent advances in wood technology, in North America, have raised the status of poplars from "weed trees" to one of importance. New products for which poplars are suitable, and even preferred to other tree species, permit industry to capitalize on the exceptionally high fiber-producing capacities of these trees. Furthermore, there is the prospect, based on European and North American experience in poplar breeding, that yields can be significantly increased by crossing our native species with exotic strains. Simultaneous improvements in tree form, wood quality, rooting capability, and other characteristics may also be effected. Unfortunately, genetic manipulation may accentuate a pronounced predisposition of poplars to pathological deterioration, which has always caused heavy losses. Hence, measures are required that will eliminate, or at least minimize, the depredations of destructive pathogens on existing and future poplar varieties. Since the control of forest tree diseases is usually based on the principle of interrupting the life cycle of casual organisms at vulnerable points, it is essential that the biology of any fungus exhibiting destructive capabilities or potentialities on valuable poplars should be elucidated.

Clarification of a complex of fungi which cause leaf and twig blights of poplar in North America is needed. In that complex is *Venturia populina* (Vuill.) Fabric. (= *Didymosphaeria populina* Vuill. (7)), an organism which is very injurious to certain European poplars, and which has been collected[3]

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Contribution No. 706, Forest Biology Division, Department of Forestry, Ottawa, Canada. Based on part of a thesis presented in May, 1955, to the University of Toronto in partial fulfillment of the requirements for the degree of Master of Arts.
[2]Regional Research Laboratory (Forest Biology), Maple, Ontario.
[3]Identification of *V. populina* furnished by Dr. R. F. Cain, Associate Professor and Curator, Department of Botany, University of Toronto, Toronto, Ontario.

on balsam poplar (*Populus balsamifera* L. (3, 8)) at Chalk River, Ontario. This paper deals with the recognition of this fungus in Ontario, its taxonomic relationships, and its pathology. The study was conducted at the Petawawa Forest Experiment Station, Chalk River, Ontario, and at the Southern Research Station, Maple, Ontario. The results presented here with respect to the biology and relationships of *V. populina* should prove useful both in developing measures for controlling the pathogen and in understanding the complex of fungi that cause leaf and twig blights of poplars in North America.

The abbreviation of herbaria names conforms to the third edition of the Index Herbariorum.

## The Disease

*Venturia populina* causes a tip blight of balsam poplar that is recognizable at any time of year. When in the leafless condition, the crowns of affected trees appear closed, stunted, and decadent. The closed character is caused by the crowding of numerous adventitious shoots and branchlets which have arisen from infection in the terminals. The decadence is attributed to canker-forming and decay-causing secondary organisms which have entered the dead shoots. The stunting probably is the result of continuing annual reductions in leaf photosynthetic area by the infections. In newly infected saplings, the principal disease symptom is the large number of blighted, reflexed shoots or "shepherd's crooks" (Fig. 1). Since infections of *V. populina* seem to persist for only a single season, perennial reinfection must occur to ensure perpetuation of the fungus. Infections are of two kinds, primary and secondary, according to the type of inoculum, tissues affected, and disease signs and symptoms developed.

Primary infections are initiated by ascospores of *V. populina* ejected from perithecia which develop on overwintered, blighted, balsam poplar shoots. In the Chalk River area these infections usually appear by the third week in May on whorls of leaves that have emerged from winter buds subjacent to the dead, perithecia-bearing part of the diseased terminal shoots (Fig. 7). The tip of each leaf in each whorl is curled, black, brittle, and truncated (Fig. 9), whereas healthy leaves have acute or short-acuminate tips. Close inspection usually discloses the presence of the imperfect stage of the fungus as a compact, olive-brown, conidia-bearing layer overlying the necrotic tissues (Figs. 11, 12, 14).

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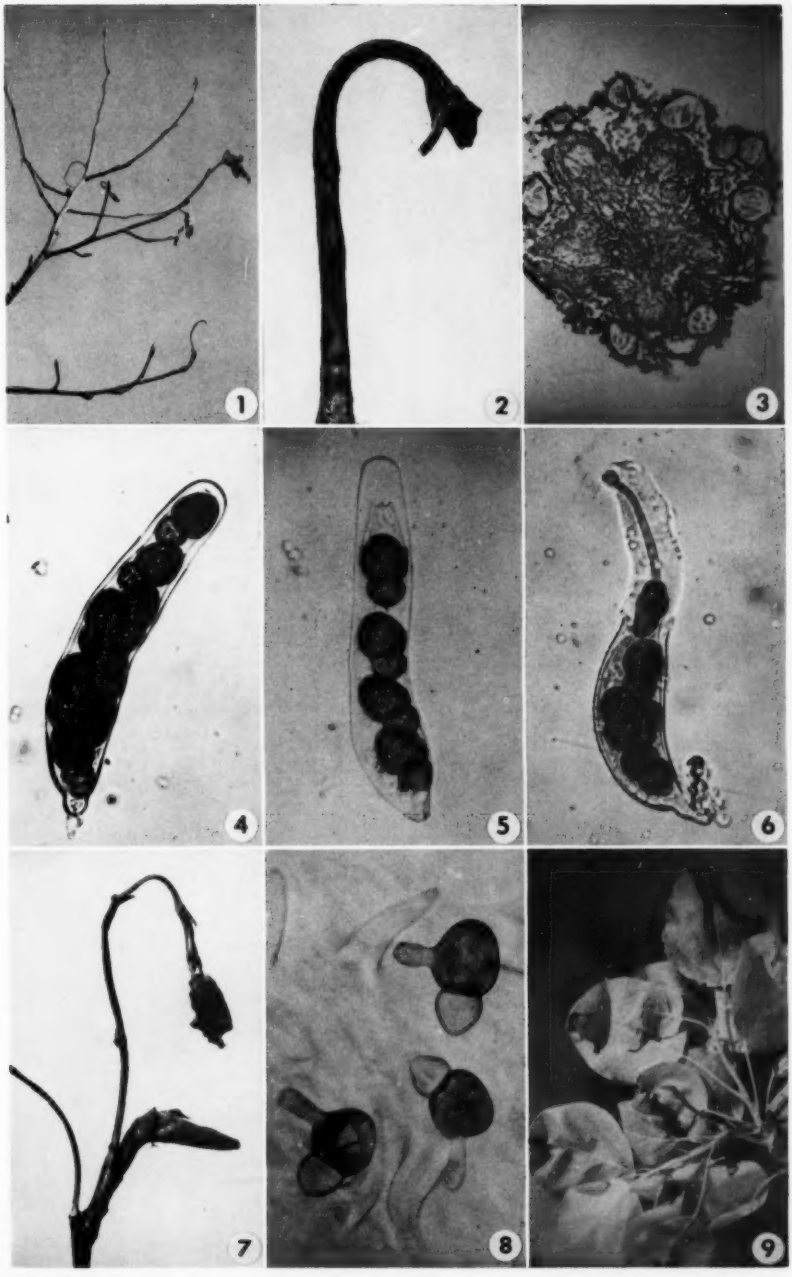
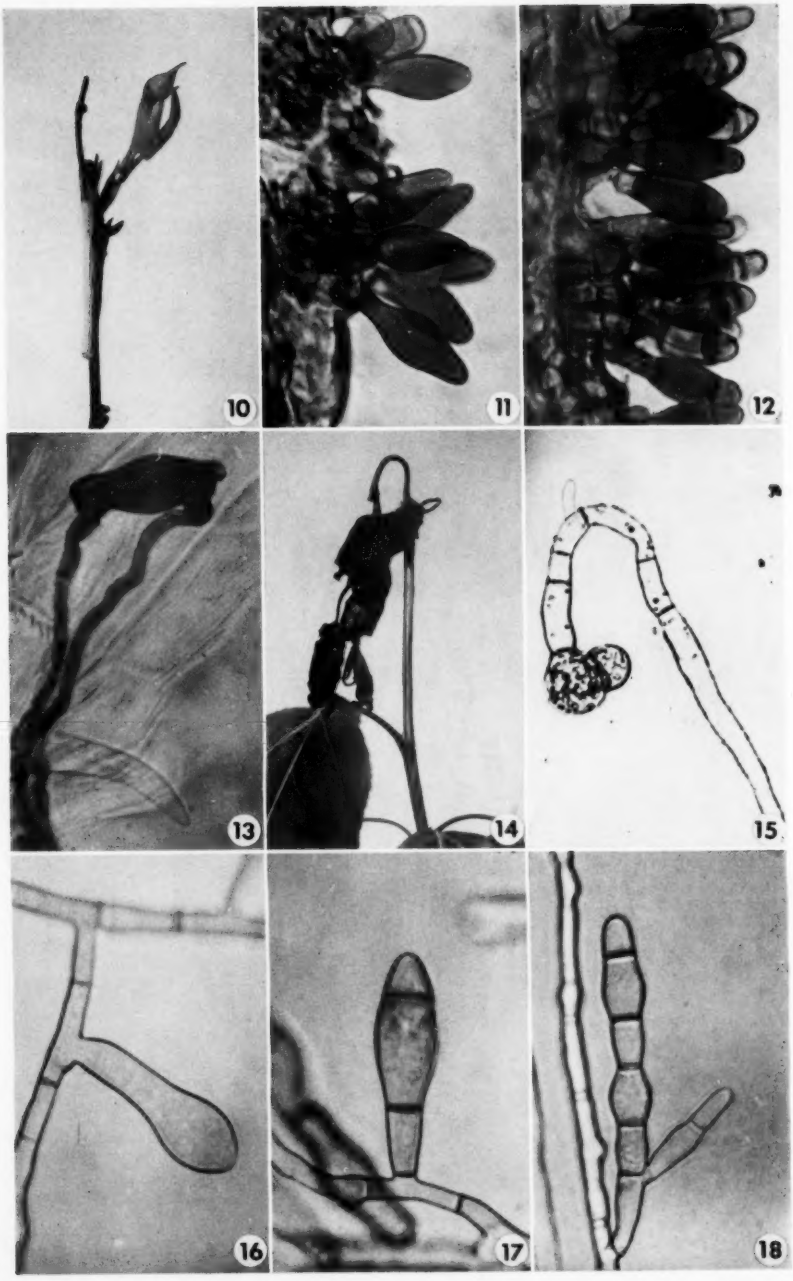
*Venturia populina* on balsam poplar: FIG. 1. Dormant, diseased sapling retains blighted, perithecia-covered leaves following autumn defoliation (×1/10). FIG. 2. Blighted twig bearing many perithecia which appear as black dots (×2). FIG. 3. Transverse, freehand section through a blighted twig: perithecia are partially embedded in the cortical and epidermal tissues of the host (×30). FIG. 4. Typical eight-spored ascus (×500). FIG. 5. Rare, four-spored ascus (×500). FIG. 6. Ascus ejecting spores: three spores have been discharged; a fourth is being extruded through the neck of the protruded inner wall (×500). FIG. 7. Both the vertical, blighted shoot and the overwintered leaf bear perithecia; ascospores from these sites may inoculate leaves emerging from the subjacent bud. The lowermost twig curving to the left bore perithecia in the preceding year (×1). FIG. 8. Germinating ascospores; germ tubes turn downward and, assisted by an appressorium (here invisible), penetrate the epidermis directly (×650). FIG. 9. Primary infections at the apices of leaves developed in a whorl from a winter bud; the source of primary inoculum was the blighted twig at the center of the picture (×1/3).




Dance—Can. J. Botany




Dance—Can. J. Botany

Secondary infections first appear early in June on the leaves and towards the tips of most new shoots arising from, or below, whorls of leaves with primary infections. They are started by conidia from primary infections. The diseased tissues turn black and brittle while the shoots reflex to form characteristic crooks, with the necrotic areas soon becoming covered with a thin, conidia-bearing layer of the fungus (Fig. 14). Shoots and leaves produced subsequently also may be subject to conidial inoculation and infection; if so, the resulting signs and symptoms are indistinguishable from those that appear earlier. While secondary infections may develop on apparently mature leaves of water sprouts and suckers, none will be found on the blades of mature leaves that bear primary infections at their tips.

By late August, immersed, immature perithecia are present in the tissues of leaves and shoots killed during the growing season. However, the perithecia do not mature and become erumpent until several months later[4] (Figs. 2, 3, 7).

The symptoms of the blight caused by *V. populina* in Ontario are similar to those described for this disease in Europe (15, 21), where it is much more prevalent and destructive.

## The Causal Organism

### *1. Isolations*

Cultures of the pathogen were consistently obtained from ascospores, from mycelia in necrotic tissues, and from conidia produced on these tissues. The isolation of single ascospores was accomplished as follows: blighted twigs of balsam poplar bearing mature perithecia were collected at Chalk River in March and were cut into sections 3–5 cm long. These sections were soaked briefly in water and fastened inside the lids of inverted petri dishes containing malt agar above and water below. Ascospore ejection commenced about 20 hours later and continued for about 40 hours. Approximately 10% of the spores which adhered to the agar surface germinated (Fig. 15). These were picked off and transferred to individual malt agar slants. Minute colonies were visible 5 days later. The production of conidia, which had occasionally begun by that time, increased as the colonies aged and enlarged.

[4]Ascospore ejection and germination were induced in the laboratory in November.

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*Pollaccia elegans* on balsam poplar and in culture: FIG. 10. Infection resulting from artificial inoculation on a young leaf; ascospores were shot from perithecia on the blighted twig to leaves emerging from the bud. The blighted twig was fastened to the healthy shoot to simulate the natural arrangement of these structures (×3/4). FIG. 11. Young, single-celled conidia on a diseased leaf. Transverse, freehand section (×600). FIG. 12. Clusters of mature, three-celled conidia on a thin, erumpent, intraepidermal stroma on a blighted leaf; the abbreviated, indistinct conidiophores are difficult to record photographically. Transverse, freehand section (×600). FIG. 13. Conidium germinating from both end cells, on a leaf (×750). FIG. 14. Typical secondary infection; the shoot has curved into the characteristic crook, the upper surface of each dead, hypotrophied leaf is covered with a layer of conidia. In early June (×2/3). FIG. 15. Initiation of a colony on agar, from a germinating ascospore of *V. populina*; as usual, the germ tube emerges from the larger cell (×700). FIG. 16. Early stage in conidium formation; a hyphal branch has enlarged towards its apical end (×1500). FIG. 17. Two-celled conidium which probably will separate from the single-celled conidiophore at the septum; in the conidium, another septum is likely to develop near the base. On agar (×1100). FIG. 18. Unusual conidial formation; the lowermost, two-celled spore probably formed after the three-celled, terminal conidium. Lateral proliferation of a third conidium is occurring. On agar (×600).

Cultures were obtained from mycelia in blighted leaves and shoots by the following procedure: small sections of the affected tissues were surface-sterilized in dilute solutions of commercial "Javex" (sodium hypochlorite), rinsed in distilled water, plated on malt agar, and incubated. The cultures which developed were indistinguishable from those which were initiated by single ascospores.

Cultures were derived from naturally occurring conidia on blighted tissues by preparing aqueous suspensions of the spores and dispersing the suspensions on malt agar. After 20 hours single conidia which had germinated were removed to individual malt agar slants. The resultant colonies, including the conidia they produced, were indistinguishable from colonies obtained by either of the former procedures.

These results confirm the connection between *V. populina* and the hyphomycete on blighted balsam poplar leaves and shoots, which had been suggested by the constant association of fruiting structures of both stages and by morphological criteria.

Although it had been reported that the perithecia of this fungus have been produced in culture (10, 12, 19), none were so developed during this study. In attempts to induce the formation of perithecia in culture, the fungus was grown on malt agar and on infusions of powdered balsam poplar leaves in malt agar, with the cultures being maintained at temperatures ranging from subzero to 21° C for more than a year. Structures appeared which may have been perithecial initials, but they did not give rise to perithecia.

*2. Identification*

The hyphomycete on balsam poplar was identified as *Pollaccia*[5] *elegans* Serv. by comparing it with the description of *P. elegans* (19) and with cultures grown from transfers[6] of the original cultures used by Servazzi in describing that fungus. These comparisons showed that the hyphomycete from Ontario agreed: (i) with the description of *P. elegans* in symptomatology, morphology, cultural characters, and to a certain extent in host range; and (ii) with the cultures of *P. elegans* in all major respects, although a few differences were noted in growth rates (slightly lower in transfers of Servazzi's cultures) and in the prevalence and size of hyphal inclusions. Perhaps these discrepancies can be attributed to changes in Servazzi's cultures induced by successive transferring since 1938, to the influence on the fungus of differing environments in Europe and North America, or to the existence of slightly different strains of the fungus on the two continents.

## Pathogenicity Trials and Elucidation of the Susceptibility of Different Poplar Species to *V. populina*

The pathogenicity of *V. populina* was tested by artificially inoculating three species and one variety of *Populus* which represented three different taxonomic sections of that genus.[7] These were balsam poplar in the section *Tacamahaca* Spach; Lombardy poplar (*Populus nigra* L. var. *italica* Muenchh.)

[5]Genus described by Baldacci and Ciferri (1).
[6]Obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland.
[7]Systematic positions herein are according to Rehder (17).

in the section *Aigeiros* Duby;[8] trembling aspen (*P. tremuloides* Michx.), and large-toothed aspen (*P. grandidentata* Michx.), both in the section *Leuce* Duby. The inocula were ascospores from perithecia on overwintered, blighted twigs (Fig. 2), and conidia from sporulating, secondary infections on balsam poplar leaves (Figs. 12, 14).

## I. Inoculations with Ascospores

### *In the Laboratory*

The material to be inoculated consisted of healty, dormant, 10-in. cuttings of balsam poplar, Lombardy poplar, and trembling aspen, collected in March at Chalk River and near Maple, Ontario. These were surface-sterilized (22) with "Semesan" (hydroxymercurichlorophenol), potted, and left at room temperature (21° C) for about one week until leaves and, in balsam and Lombardy poplars only, roots were developing. Thirty pieces of perithecia-bearing twigs were surface-sterilized in dilute "Javex" and washed in distilled water. Each piece was then fastened to a leaf-bearing shoot, about 1.5 cm from a whorl of young leaves (Fig. 10), in a position and attitude simulating the natural relationship noted between overwintered, blighted shoots and subjacent whorls of healthy leaves, or leaf buds (Fig. 7). This arrangement was replicated 10 times for each tree species. The potted shoots were placed in sealed glass aquaria, each holding 2 cm of water, to maintain leaf turgor and the high relative humidity shown by tests to favor ascospore discharge and germination. To further stimulate ascospore ejection, the perithecia were sprayed with water then and daily thereafter for 4 days. Thirty controls were established by selecting 10 separate whorls of healthy leaves of each poplar species comparable to the inoculated whorls, but not opposite pieces of perithecia-bearing twigs. To determine if spores had been shot to exposed leaves during the 4-day interval, a piece of leaf tissue, 5 × 5 mm, was cut from one leaf of each poplar species in that region of the blade opposite a piece of perithecia-bearing twig. The samples were cleared in boiling lacto-phenol and examined microscopically. In every sample a high concentration of ascospores was noted on that epidermal surface that had faced perithecia. In the samples from balsam and Lombardy poplars, about 80% of the spores had developed germ tubes which formed appressoria and had penetrated the epidermal cells directly (Fig. 8). These germ tubes were squat and uniform in diameter. On trembling aspen only about 10% of the spores germinated, and these produced germ tubes which grew erratically over the epidermal surface without penetrating it; they were etiolated, irregular in diameter, and soon collapsed or disintegrated. Ungerminated spores also collapsed or disintegrated; in this respect they were unlike ungerminated spores on balsam and Lombardy poplars, which generally retained their original form.

Four days later, brown spots averaging about 1 cm in diameter developed opposite the pieces of perithecia-covered twigs on the inoculated leaves of balsam and Lombardy poplars; but none appeared on trembling aspen leaves or on the leaves of any species designated as controls.

About this time, most of the inoculated leaves and controls on shoots of

[8]Section *Aigeiros* Duby (6, 9); but, section *Aegeiros* Duby (17).

trembling aspen developed various-sized necrotic areas which later coalesced, thereby masking the inoculations and preventing completion of this aspect of the infection studies. The cause of this condition remains unexplained although it may be physiogenic and the result of the aspen cuttings failing to root. One week later the spots on balsam poplar had become heavily sporulating infections, faintly resembling small, natural, secondary infections (Fig. 10). However, the spots on Lombardy poplar failed to sporulate then or subsequently. No necrotic areas or conidia like those on balsam poplar developed on the controls of any species.

*In the Field*

In early May branches and branchlets of balsam poplar with perithecia-covered shoots were collected at Chalk River, compressed into bundles, and tied in the healthy crowns of balsam poplar, Lombardy poplar, trembling aspen, and large-toothed aspen at two locations near Maple (it had been determined, in the preceding year, that these particular trees were not infected with *V. populina*). The trees were examined for the presence of infections in early June; on balsam poplar, numerous, small, sporulating, necrotic spots were observed on leaves[9] adjacent to and especially below the bundles of twigs. All the infections were 5 mm or less in diameter. No blighted shoots were observed. The exposed crowns of Lombardy poplar, trembling aspen, and large-toothed aspen showed no symptoms of infection. Although all the trees were re-examined several times subsequently, no reinfections were observed on balsam poplar, and the crowns of the other three species remained uninfected.

## II. Inoculations with Conidia

Inoculations with conidia of *P. elegans* were made on balsam poplar and trembling aspen in the laboratory and in the field at Chalk River.

*In the Laboratory*

In June, healthy, 10-in. cuttings of both poplar species bearing semimature leaves were surface-sterilized in 80% methyl alcohol, rinsed, potted, and placed in glass moist chambers, as before in ascosporic inoculations. A highly concentrated conidial suspension was brushed on 10 leaves, mostly on separate shoots, of both poplar species. Controls were established by painting distilled water on 10 leaves of both tree species, on shoots with inoculated leaves. All leaves were sprayed daily with water to stimulate spore germination. Four days later, sample sections of inoculated leaves were removed and processed for microscopic examination. It was observed that: (i) on balsam poplar, most conidia had germinated (Fig. 13) and developed rather profuse, superficial mycelia which occasionally had sporulated without causing necrotic spots; and (ii) on trembling aspen, only a few conidia had germinated—these had usually produced some superficial, distorted hyphae which eventually collapsed without sporulating or causing spots. The potted cuttings were then removed from the moist chambers and placed outdoors in a shaded

[9]Sample leaves deposited in the herbarium (MFB-5399); Regional Research Laboratory, Maple, Ontario.

location to encourage the development of any incipient infections. As before with ascosporic inoculations, most of the leaves on the aspen cuttings soon developed necrotic areas that were not attributable to infections but which terminated the usefulness of the tests on this host. Subsequent inspection of the inoculations and controls on balsam poplar failed to reveal typical infections of *P. elegans*.

*In the Field*

In July, 10 healthy, adult, balsam poplar leaves and 10 of trembling aspen, all on separate shoots, were inoculated *in situ* by painting a concentrated conidial suspension on their upper and lower surfaces. To facilitate germ tube penetration, the axial surface of each leaf was first lightly abraded with 500-mesh carborundum powder. Twenty controls were similarly established; however, in these, distilled water was substituted for spore suspension. Each treated whorl, including an inoculated and a control leaf, was enclosed in a waxed-paper bag containing a moist pad of absorbent cotton. Examination of inoculated leaves, processed 4 days later by the excision method used previously, revealed that numerous conidia had germinated on balsam poplar leaves and a few on leaves of trembling aspen. However, no visible infection appeared then or subsequently on any inoculated or control leaf.

## III. Discussion of Results

The consistent development of sporulating infections on young balsam poplar leaves inoculated with ascospores of *V. populina* identified this ascomycete as the cause of the disease on balsam poplar, and confirmed the identity of the hyphomycete on blighted balsam poplar leaves, already indicated by other criteria, to be *P. elegans*.

The failure of artificial conidial inoculations to start infections on balsam poplar leaves is attributed to the adverse influence of undetermined environmental factors and to ability of mature and semimature leaves and shoots to resist infection, although variation in clonal resistance may also be a factor.

Evidence supporting the role of environmental factors seemed to be present in the sporadic distribution of *P. elegans*. Whereas the incidence of the fungus in one group of trees was high, discontinuous groups of trees in the same vicinity remained uninfected. This discrepancy may indicate failure of ascospores and conidia to survive transmission through adverse environmental conditions prevailing between their developmental site and the healthy, discontinuous groups of trees; the existence of environmental conditions unfavorable to infection in the healthy groups; or a combination of these factors. That one group of diseased trees can be perennially reinfected may be attributed to the recurrence of favorable, but strictly local, environmental conditions which persist for periods of sufficient duration to allow initiation and establishment of infections.

Evidently, balsam poplar leaves and shoots develop resistance as they mature. This conclusion is drawn from certain peculiarities of natural infections mentioned earlier, and from the fate of conidia brushed on older leaves in the artificial inoculations, namely: (i) only young leaves or shoots are

killed by infections that appear in June or later, that is, by secondary infections; leaves of adult size which become infected, then, have juvenile characteristics due to their origin on adventitious and sucker shoots; (ii) infections, started by ascospores, that appear at the apices of leaves in May, do not spread into the mature, healthy tissues of the blade; (iii) new infections do not develop on the blades of such leaves, despite the proximity of copious inoculum to healthy leaf tissue; and (iv) although artificially induced infections were started with ascospores on young leaves, none developed from conidial inoculations on older leaves notwithstanding a high rate of spore germination, vigorous germ tube development, and substantial growth of superficial mycelium.

It may be concluded that, while the resistance of mature balsam poplar tissues cannot prevent the occurrence of infections of *V. populina* and deterioration in the vigor of the trees, it undoubtedly does enable diseased trees to survive indefinitely by preventing established infections from completely invading the remaining healthy leaf tissues.

Regarding the aggressiveness of *V. populina*, it was found that under the environmental conditions existing in moist chambers, the ability of the pathogen to cause disease varied with different poplar species as follows: balsam poplar was susceptible; Lombardy poplar was resistant; and trembling and large-toothed aspens seemed immune.

The failure of artificial ascosporic inoculations to induce sporulating infections on leaves of Lombardy poplar in the laboratory, or visible infections on this host in the field, is puzzling considering that in Europe this poplar variety is very susceptible to *V. populina*. If the fungus has been introduced to North America (a possibility indicated by its sporadic distribution and, at Chalk River, its proximity to introduced poplars) then one explanation for the seminegative results obtained is that this fungus has lost its aggressiveness on Lombardy poplar in the process of adapting to North American hosts and environmental conditions.

## The Fungus

### I. Ascigerous State, *Venturia populina* (Vuill.) Fabric.
(Loculoascomycetes, Pseudosphaeriales, Venturiaceae)

On a typical blighted shoot of balsam poplar, perithecia of *V. populina* occur mainly along the curved inner surface of the crook induced by shoot blight and immediately below leaf scars (Figs. 2, 3, 7). On a leaf, mature perithecia are erumpent and clustered about the midrib and larger veins on both surfaces (Fig. 7), and are particularly prevalent on the petiole. The perithecia are globose, smooth, brownish-black, averaging 0.2 mm in diameter and contain about 20 asci. Asci nearly sessile, 96–124 × 21–24 $\mu$ (85 × 28 $\mu$ according to Vuillemin (21)), usually eight-spored (Fig. 4), rarely four-spored (Fig. 5). Ascospores 21–30 × 10.5–16 $\mu$, olive-brown, smooth, unequally two-celled, strongly constricted at the septum, the larger cell rounded, the smaller cell tapered (Fig. 8). Pseudoparaphyses slender, colorless, evanescent prior to ascospore discharge.

Ascospore discharge in water was witnessed occasionally: the eight spores were shot in rapid succession, in a second or less (Fig. 6), the violence of

ejection propelling them about 0.5 cm, through the water—the probable equivalent of several centimeters through air.[10]

### II. Conidial State, *Pollaccia elegans* Serv.

*On the Host*

Conidiophores short, zero-, one-, or two-celled, solitary, in pairs or clusters of three or more, erect on a thin, black, intraepidermal stroma in necrotic host tissue (Figs. 11, 12) characteristic of the genus (13), mostly on the axial surface of leaves. Conidia solitary at tips of conidiophores, unicellular when young, usually three-celled later, frequently two-celled and, occasionally, four- or five-celled, greenish- or golden-yellow at first, deep olive-brown later, truncate at distal end of basal cell, longitudinally symmetrical, 16–42 × 8–14 μ.

*In Culture*

Colonies even, greenish-black, slow-growing (diameter 11 mm after 2 months on malt agar at 21° C); mat raised, velvety, thin, uniform throughout. Hyphae thin-walled, dark, with numerous, large, round swellings, lateral protuberances, and small, variable-sized, oily inclusions. Numerous, black, pseudoparenchymatous cells present. Conidiophores sporadic outgrowths of hyphal walls, submerged in agar (Fig. 16), zero- or one-celled (Fig. 17). Determinate type conidial development; usually only one conidium per conidiophore, but occasionally two or more (Fig. 18). In most other respects, as described "on the host".

### III. Spermatial State

In 1889, Vuillemin described (21) *Phoma populina* Vuill.,[11] which he believed to be the sole imperfect stage of *V. populina*. Recently, however, Servazzi contended (19) that *P. populina* does not constitute part of the life cycle of *V. populina*.

During the course of this investigation, an unidentified species of *Phoma* with pycnidiospores larger than those of *P. populina* was found on blighted twigs of balsam poplar bearing perithecia of *V. populina*. However, it could not be determined if this spermatial stage constitutes part of the life cycle of *V. populina*, because the pycnidiospores did not germinate either in water or on malt agar.

## A Comparison of *Pollaccia elegans* and *Pollaccia radiosa*

In 1959 *Pollaccia radiosa* (Lib.) Bald. & Cif. was connected with *Venturia tremulae* Aderh. in Ontario (4). Previously, this ubiquitous and destructive hyphomycete on aspen had been regarded in North America as the imperfect stage of *D. populina* (14). Accordingly (15), it had been called *Napicladium tremulae* (Frank) Sacc. (= *P. elegans* (19)). The confusion created by this error was subsequently compounded by the application to this hyphomycete of several other names, some of which, according to Servazzi (19), are synonymous with *P. elegans* (for instance, *Fusicladium radiosum* (Lib.) Lind), while

[10]According to Clark (2), the viscosity of: (1) water at 0° C is 0.01787 dynes per sq. cm; (2) air at 0° C is 0.000171 dynes per sq. cm.

[11] Descriptions of *Phoma populina* and *Didymosphaeria populina* recorded by Saccardo (18).

TABLE I

Field characters of *Pollaccia radiosa* and *Pollaccia elegans* in Ontario

| Character | *P. radiosa* | *P. elegans* |
|---|---|---|
| Host range (*Populus*) | Section *Leuce* Duby | Section *Tacamahaca* Spach |
| Prevalence | Prevalent throughout range of the host | Scarce; affected trees observed only occasionally and then in small, local concentrations |
| Size of trees affected | Infections confined mostly to regeneration and saplings | Infections occur irrespective of the size or age of the host |
| Distribution on host | Mostly on terminal leaves and shoots | Heavy infections occur both on terminal and lateral shoots and leaves and throughout the entire crown |
| Types of infection | Primary and secondary infections indistinguishable | Primary and secondary infections distinct respecting tissues affected, symptoms, and time of appearance |
| Duration of sporulation | May continue until early October depending upon host and climatic conditions | No sporulation after mid-July |

TABLE II

First recognized Canadian collections of *Venturia populina* and *Pollaccia elegans*

| Accession No. | Host | Locality | Name applied |
|---|---|---|---|
| MFB 2637 | *Populus balsamifera* L. | Killaloe, Ontario | *Venturia populina* (Vuill.) Fabric. |
| MFB 2638 | *P. balsamifera* | Chalk River, Ontario | *V. populina* |
| MFB 2639 | *P. balsamifera* | " " " | *V. populina* (*Pollaccia elegans* Serv. also present) |
| MFB 2640 | *P. balsamifera* | " " " | *P. elegans* (primary infections) |
| MFB 2641 | *P. balsamifera* | " " " | *P. elegans* (secondary infections) |

others are synonyms of *P. radiosa* (for example, *Fusicladium tremulae* Frank). Because of the confusion which has existed respecting the identity of the imperfect stages of *V. populina* and *V. tremulae*, it seems worth while to record, in Table I, the more conspicuous differences between the diseases they cause as an aid to their identification in the field.

## North American Collections of *Venturia populina* and *Pollaccia elegans*

In 1951 collections of *V. populina* and its imperfect stage *P. elegans* were made on balsam poplar at Chalk River. Additional collections of the ascigerous stage were obtained subsequently at Killaloe, Ontario. Particulars respecting these collections are recorded in Table II. To ascertain whether these constituted the first North American records of this fungus, records and collections of fungi on *Populus* in the literature and in three ranking North American herbaria were examined.

*1. Canadian Records*

No Canadian records of *V. populina* or of *P. elegans* were found either in the literature or the herbaria. However, one collection of the ascigerous stage (DAOM-3218) and six of the imperfect stage (DAOM-3218, 2951, 38208, 3601, 18300, and 38233) were found deposited under other names. The single collection of *V. populina* probably had been overlooked because of heavily sporulating infections of *P. elegans* present in the sample. Particulars respecting these collections appear in Table III.

Each collection of *P. elegans* had been originally identified either as *Fusicladium radiosum* or, once (DAOM-3601), as *F. radiosum* (Lib.) Lind. var. *balsamiferae* J. J. Davis. Therefore, they were correctly named because *F. radiosum* is synonymous with *P. elegans* (19), and, as will be shown, *F. radiosum* var. *balsamiferae* is also a synonym of *P. elegans*. However, these collections of *P. elegans* constituted only a small fraction of all the collections from poplar that had been initially identified as *F. radiosum*. Since most of the collections so identified were recognized by the author to be actually *P. radiosa*, it seemed that *P. elegans* had been confused with *P. radiosa* which, as pointed out previously, had been erroneously regarded as the imperfect stage of *Didymosphaeria populina*. This probability was confirmed by the different host ranges encountered in the collections of *P. elegans* and *P. radiosa*. These agreed with Servazzi's conclusions (19) and with the results of artificial infection trials in this study that the host ranges of *P. elegans* and *P. radiosa* are confined to different taxonomic sections of the genus *Populus*; namely, whereas all collections which represented *P. elegans*, except DAOM-38233 where the host was unidentified, were from poplar species of the section *Tacamahaca*, all collections that represented *P. radiosa* were from poplar species of the section *Leuce*. It was concluded that although the collections of *P. elegans* in Table III bore suitable epithets, this probably was accidental.

DAOM-3601, in Table III, was originally identified as *F. radiosum* var. *balsamiferae*; however, this collection clearly represents *P. elegans* because it fits the description of that hyphomycete, and because the host is balsam poplar in this instance, in the type collection (5), and in the single record listed by Seymour (20), that is, in every record encountered in this study.

TABLE III

Herbarium specimens of poplar pathogens renamed *Venturia populina* and *Pollaccia elegans*

| Accession No. | Host | Locality | Herbarium designation |
|---|---|---|---|
| Ottawa | | | |
| DAOM 3218* | *Populus balsamifera* L. | St. Gabriel, Que. | *Fusicladium radiosum* (Lib.) Lind |
| DAOM 2951 | *P. balsamifera* | Edmonton, Alta. | *F. radiosum* (Lib.) Lind |
| DAOM 38208 | *P. balsamifera* | Kananaskis, Alta. | *F. radiosum* (*Pollaccia radiosa* (Lib.) Servazzi) |
| DAOM 3601 | *P. balsamifera* | Fort St. James, B.C. | *Fusicladium radiosum* (Lib.) Lind var. *balsamiferae* J. J. Davis |
| DAOM 18300 | *Populus tacamahaca* Mill.? | Bolton Centre, Que. | *F. radiosum* |
| DAOM 38233 | *Populus* sp.? | ? | *F. radiosum* (= *N. tremulae*) |
| University of Toronto | | | |
| TRTC 832† | *Populus trichocarpa*‡ Torr. and Gray | Mt. Hood Loop Highway, Oregon | *F. radiosum* |
| Beltsville | | | |
| BPI unnumbered | *P. trichocarpa* | Mt. Hood Loop Highway, Oregon | *F. radiosum* |
| BPI 808 | *Populus* sp. | Iowa | *Napicladium tremulae* (Frank) Sacc. (= *F. radiosum*) |

*The only collection in which *V. populina* was encountered.
†Apparently part of BPI—unnumbered.
‡A western species frequently mistaken for balsam poplar (3).

*2. American Records*

*A. In herbaria.*—As in the case of Canadian herbaria, no collection identified as *V. populina* or *P. elegans* from the United States had been recorded in any of the three herbaria, nor was the ascigerous stage detected among fungal collections on poplar deposited in those herbaria. However, *P. elegans* was recognized in three collections (TRTC-832; BPI—unnumbered, labelled *F. radiosum*; and 808: apparently TRTC-832 forms part of BPI—unnumbered). The remaining collections, mostly labelled *F. radiosum*, were recognized as *P. radiosa*. It was noted that the hosts in these collections of *P. elegans* either belonged, or probably belonged, to the section *Tacamahaca*; whereas in the collections of *P. radiosa* the hosts were, apparently, confined to the section *Leuce*.

*B. In the literature.*—Because check lists of fungi on *Populus* (20, 23, 24, 25) include numerous records of *D. populina* (= *V. populina*) and *N. tremulae*, it appeared at first that *V. populina* had been frequently collected. It is clear, however, from synonymy in those indices, that synonyms which are correct for *V. populina* and *P. elegans* have been used interchangeably and indiscriminately with synonyms which are correct only for *V. tremulae* and *P. radiosa*. Therefore, the author is skeptical of any reports of *V. populina* in North America which are based on collections from hosts not belonging to the section *Tacamahaca*. However, despite the total absence of collections,[12] the possibility of its occurrenee here on species of the section *Aigeiros* cannot be discounted entirely because of the European experience with the blight on these hosts.

*3. Summary*

Although host material bearing *V. populina* (and *P. elegans*) had been collected in Canada prior to this investigation, the ascigerous stage had not been detected and the imperfect stage apparently had been confused with *P. radiosa*. Hence, the collections made at Chalk River seem to constitute the first correctly identified Canadian records of the fungus. Furthermore, because it is unlikely that either stage had been correctly identified in the United States, they probably constitute the first correct North American records of the fungus. *Fusicladium radiosum* (Lib.) Lind var. *balsamiferae* J. J. Davis should be regarded as a synonym of *Pollaccia elegans* Serv.

## Control

Since *V. populina* occurs only sporadically on native poplars in North America, its economic significance at present is probably negligible. However, if this pathogen should become locally destructive on ornamental poplars, as a result of a favorable combination of environmental factors, it probably could be controlled by eliminating the source of primary inoculum through pruning of blighted shoots, as recommended by Prillieux (16). The

[12] A reputed collection of *D. populina* on *P. nigra* var. *italica* (section *Aigeiros*) was later identified by T. T. Ayers as *V. tremulae*, according to information supplied in correspondence by Dr. W. W. Diehl, Mycologist, U.S. Department of Agriculture, Division of Mycology and Disease Survey, Beltsville, Md. Furthermore, this material is now in poor condition and valueless for study of the *Venturia*, according to information supplied in correspondence by Dr. A. M. Waterman, Pathologist, U.S. Department of Agriculture, Division of Forest Pathology, New Haven, Conn.

most serious danger from *V. populina* probably lies in the threat it poses to hybrid poplars whose parentage includes susceptible, exotic species or varieties. Forest tree breeders should consider this particular hazard in selecting material for their crosses.

## Conclusion

In addition to being a contribution to mycological knowledge, the findings of this study have improved the prospect of controlling leaf and twig blights of poplars. It has been shown that (1) two pathogens with distinct host preferences exist, instead of one as formerly believed; and (2) spores of *V. populina* must reach leaves at an early stage of their development in order to induce the disease. This indicates a favorable point for beginning fungicidal treatment.

It was observed that *V. populina* has a mechanism which might cause infections to develop despite efforts of tree breeders to evolve resistant or immune strains of *Aigeiros*-type poplars. This mechanism has two components: (i) inoculation of the leaves while they are susceptible; and (ii) deposition of a heavy concentration of primary inoculum on the leaves—a condition that may be conducive to spore germination (11). Secondary infections seem assured by the plentiful secondary inoculum constantly present until late July and, therefore, available for the infection of the tender, susceptible leaves and shoots which continuously arise throughout the growing season.

The first component depends upon coincidence of ascospore ejection with flushing of the leaves from winter buds and their elongation to 2–3 cm (established when ascospores were observed, in the field, on recently emerged leaves in the immediate vicinity of perithecia-bearing twigs).

The second component relies on a combination of the following four factors: (i) the presence of substantial primary inoculum resulting from the large number of blighted, perithecia-laden shoots in the crowns of diseased trees, and from the profusion of perithecia on those shoots; (ii) the proximity of primary inoculum to young leaves—there is no doubt that the consistently narrow gap between perithecia and young leaves favors inoculation by minimizing spore dispersal; (iii) the violence of ascospore ejection—the high velocity of ejected ascospores ensures their impingement on the sticky, resin-coated, adjacent leaf surface; and (iv) the duration of ascospore discharge—tests indicated that perithecia eject spores for about 40 hours and that, under favorable conditions, most spores germinate within 40 hours of their expulsion. Therefore, since most spores will have germinated within 80 hours of the inception of spore discharge, germination must occur almost exclusively on susceptible tissues. Consequently, by preventing germination from proceeding uselessly on resistant tissues, the short duration of spore discharge in *V. populina* probably increases the effective concentration of inoculum on leaves.

In the section "North American Collections of *Venturia populina* and *Pollaccia elegans*", it was concluded that *Fusicladium radiosum* (Lib.) Lind var. *balsamiferae* actually represents *P. elegans*. That Davis (5) did not recognize his type collection to be the imperfect stage of *V. populina* is understandable considering that: (i) *V. populina* was not present in that collection;

(ii) prior to 1920 *V. populina* had never been observed in North America (14) nor had it been recorded by 1922 when *F. radiosum* var. *balsamiferae* was described; (iii) the reputed variety resembled *P. radiosa* which had been regularly confused with *P. elegans*; and (iv) *P. elegans* is scarce compared with *P. radiosa*. Indeed, considering these and other factors already discussed, such as the previously unrecognized different host preferences of *V. populina* and *V. tremulae*, the misuse of synonymy, and other misconceptions respecting these two fungi, it is remarkable that Davis discerned any basis for creating *F. radiosum* var. *balsamiferae*. On the contrary, since Servazzi did not include (19) Davis's varietal description in the exhaustive bibliography accompanying his description of *P. elegans*, it may be assumed that he was unaware of its existence, otherwise he probably would have listed *P. radiosa* var. *balsamiferae* as an additional synonym of *P. elegans*.

The North American host range of *V. populina* disagrees with its host range in Europe by, apparently, being confined to the section *Tacamahaca* rather than the section *Aigeiros*; however, both host ranges agree in excluding poplars of the section *Leuce* to which *V. tremulae* seems to be confined. These conclusions are based on the results of artificial infection studies, on the host range encountered in the first Canadian collections of *V. populina* and *P. elegans* to be recognized, in the renamed Canadian collections of both stages, in the renamed United States collections of *P. elegans*, and in Servazzi's definition (19) of the host range of *V. populina*.

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# CHANGES IN THE DRY WEIGHT, PROTEIN, NUCLEIC ACID, AND CHLOROPHYLL CONTENTS OF GROWING PEA LEAVES[1]

ROBERT M. SMILLIE[2] AND G. KROTKOV

## Abstract

Pea leaves of different ages were harvested over a 10-day growth period and were analyzed for the following: fresh weight, dry weight, cell number, chlorophyll, soluble protein, ribonucleic acid, and deoxyribonucleic acid. The chlorophyll content increased rapidly during early leaf expansion, then remained fairly constant. Only small differences were found in the percentage of soluble protein in the leaves during their development. In contrast, the ribonucleic acid and deoxyribonucleic acid levels in the leaves decreased continuously as the leaves aged. These results are discussed in relation to similar analyses performed on leaves from other higher plants.

## Introduction

The physiological development of leaves as influenced by environmental changes has been the subject of numerous studies. Most frequently, the physiological changes accompanying leaf growth have been ascertained in terms of variations in the morphology and composition of the leaves and in changes in metabolic processes such as photosynthesis and respiration (18). To a lesser extent, the activities of certain enzymes have been followed during the growth of leaves. However, few attempts have been made to integrate the physiological events associated with leaf development with corresponding studies at the enzymic level.

A knowledge of the biochemical processes occurring during the growth of leaves at both the enzymic and cellular levels is important for a number of reasons. Studies at both levels are necessary to establish over-all concepts of leaf growth and development and the interrelations and relative importance of the various metabolic processes contributing to this development. For example, a developmental study of photosynthesis and respiration and the enzymes participating in these events provide pertinent information on their relative roles in the energy metabolism of the growing leaf. Such studies also are important in establishing the relation of the leaf at various stages of its growth to the remainder of the plant as either an acceptor or donor of energy.

Developmental studies on leaves also have proved useful in distinguishing between enzymes involved in respiration and photosynthesis. Preliminary accounts of these studies have been published (32, 33).

A further consideration for characterizing the general metabolic variability of leaf tissue during aging is the need of reproducible material for biochemical studies. In many experiments dealing with the enzymology of leaves, considerable variations may be found in leaves of different ages. In such instances,

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the physiological age of the leaf material for the activities in question must in some way be defined. Similarly, in order to evaluate biochemical changes in leaf development induced by external environmental changes, or by introduced chemicals or infections, as much information as possible on the "normal" growth of leaves is required.

The data presented in this paper initiates a study of biochemical changes occurring during the development of pea leaves. The pea plant was chosen for this study not only because it can be easily grown and has a fairly rapid rate of growth, but also because of the fund of biochemical information which has accumulated from other studies on this plant. The pea plant has been utilized as experimental material for a wide range of experiments with the result that a sizable portion of the enzymic complement of its various tissues has been determined. Pea leaves represent one of the few instances in which mitochondria and microsomes have been isolated from photosynthetic tissues and to some extent characterized biochemically (29, 30, 31). Chloroplasts isolated from pea leaves can carry out the Hill reaction and photosynthetic phosphorylation (34). The latter has been established for isolated chloroplasts from only a few other higher plants (38).

This paper deals with compositional changes of growing pea leaves. Subsequent papers will describe various enzymic changes accompanying leaf development.

## Materials and Methods

*Plant Material*

*Pisum sativum* var. Laxton's Progress was used throughout this work. Pea seeds were soaked in water for 3–5 hours, surface-sterilized with Thioeneb 50W (Naugatuck Chemicals, Elmira, Ontario), and then sown in sterilized soil contained in wooden flats. In some experiments conducted at Brookhaven National Laboratory, Upton, N. Y., the seeds were germinated in sand contained in wooden flats. The soaking and surface sterilization of the seeds were omitted.

The pea plants were grown in steam-heated greenhouses, the temperature being maintained at 75 to 90° F. During winter, supplemental fluorescent and incandescent light was given for 16 hours per day. The plants were grown at fairly high temperatures, since our interest centered only on the vegetative growth of plants up to 2 weeks old. Further, it had been found previously that the best yields of biochemically active mitochondria were obtained from the young leaves of rapidly growing plants (31).

In order to follow changes in the growing leaves, the plants were classified arbitrarily into five stages of growth corresponding to 5- to 6-, 7-, 9-, 12-, and 16-day-old plants, respectively. In some experiments, leaves were harvested from plants of intermediate ages. All leaf samples taken for analyses consisted of two pairs of leaves detached from each plant. In order to obtain samples of sufficient weight, leaves for each sample were detached from several plants. The general appearance of representative plants at the five growth stages is shown in Fig. 1. Arrows show the pairs of leaves used for the experiments. The relative sizes of these leaves also can be seen. For convenience the five leaf samples will be referred to as stages 1 to 5 in leaf development. Ideally

PLATE I

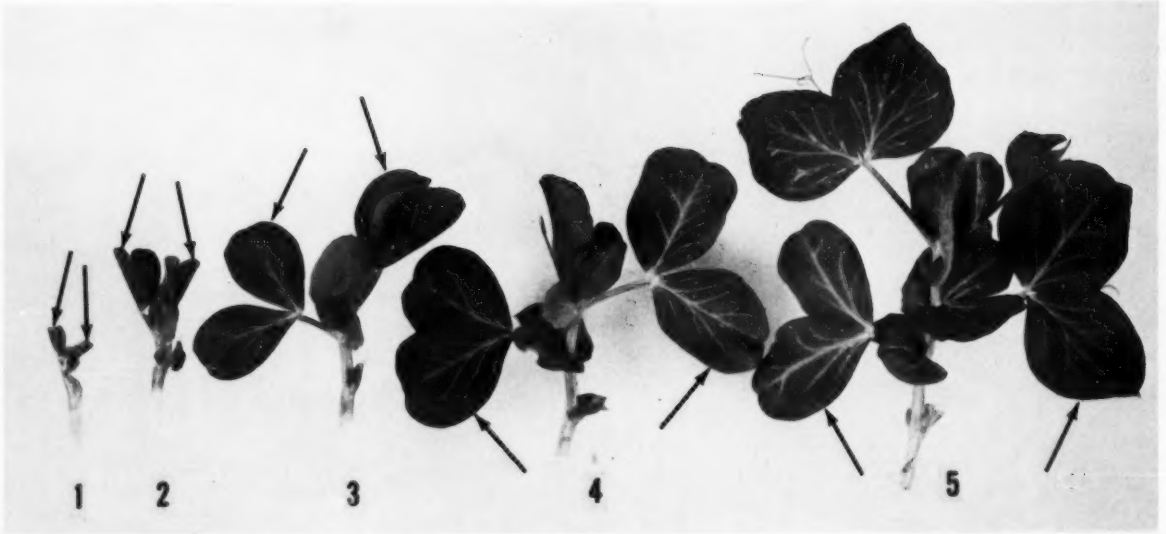


FIG. 1. Pea plants representative of the five developmental stages selected for experimentation are illustrated. The ages of the plants from germination were as follows: stage 1, 5 days; stage 2, 7 days; stage 3, 9 days; stage 4, 12 days; and stage 5, 16 days. Each pair of leaves used is indicated by an arrow pointing to one of the leaves.

it would have been preferable to confine the experiments to one pair of leaves throughout the series. Two pairs from each plant were chosen because of the large amount of leaf material required for some analyses and because of limited greenhouse space. The sampling procedure was designed to furnish samples of leaves whose average age increased throughout the series, rather than to attempt to follow the physiological development of individual leaves.

Leaves at stage 1 in leaf development (from 5- or 6-day-old plants) were harvested 1 day after the top of the plant had emerged from the soil. At this stage active chlorophyll synthesis was still progressing and the early stages in the growth series included effects due to greening in addition to growth. This method of sampling was purposely chosen so that differences between respiratory and photosynthetic mechanisms in the leaves throughout the growth series were further highlighted. Leaf expansion was generally completed by stage 4 in leaf development. The growth stages chosen thus included those of very young leaves, fully expanded leaves, and finally at the beginning of senescence.

After harvesting, the leaves were placed in Petri dishes containing moistened filter paper. Experiments were conducted on the leaves as soon as possible after detachment.

*Analytical Methods*

To determine the fresh weight of a leaf sample, the detached leaves were removed from the Petri dishes and as rapidly as possible were weighed to the nearest milligram.

For dry weight determinations, leaves were placed in a small, pre-weighed porcelain crucible and heated to 104° C for 2 days. The crucible and dried material were then weighed.

Cell count determinations were carried out according to Brown and Rickless (3). After incubation with chromic acid, the cells were evenly dispersed by moving a loosely fitting glass pestle up and down in the tube containing the cell suspension. The cells were stained with light green before counting.

Soluble protein was estimated as follows. Leaves were ground in 0.04 *M* Tris buffer, pH 7.5, using a glass pestle and mortar. The ground leaves were then transferred to a glass Ten Broeck hand homogenizer and further ground to ensure complete cell breakage. The homogenate was centrifuged at 144,000 *g* for 30 minutes to remove sedimentable material. The protein in the supernatant was precipitated by the addition of cold perchloric acid to 5% and the protein content of the precipitate estimated by the biuret reaction (9).

For the determination of total leaf chlorophyll, a leaf homogenate was prepared as described for the estimation of protein. The chlorophyll content of aliquots of the homogenate was estimated by Arnon's (1) adaptation of the method of Mackinney (17).

Nucleic acid was determined as follows. Leaf samples (5 g) were harvested and the leaves were ground in ice-cold 0.35 *M* NaCl for 3 minutes using a glass pestle and mortar. The mixture was filtered through muslin and the residue re-extracted with the NaCl solution. Nucleic acids in the combined filtrates were precipitated by the addition of ice-cold trichloroacetic acid to 5%. The mixture was centrifuged, the precipitate was washed with cold

methanol and resuspended in methanol. The samples were stored at this point in a deep freeze until required. Subsequent analyses for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were performed according to the procedure for pea leaves described by Smillie and Krotkov (35). The above procedure measured the nucleic acid of the homogenates rather than the total leaf nucleic acid. Further grinding or extraction of the ground leaves with 0.35 *M* NaCl did not increase the nucleic acid yield significantly.

## Results

*Leaf Weight and Cell Number*

Figure 2 shows the changes in dry weight and cell number per mg fresh weight for leaves at stages 1 to 4 in leaf development. The moisture content of the leaves increased with age from 82.8% at stage 1 to 87.1% at stage 4. The cell number decreased more rapidly than the dry weight, indicating that cell expansion involved the synthesis of new cell materials in addition to water uptake.

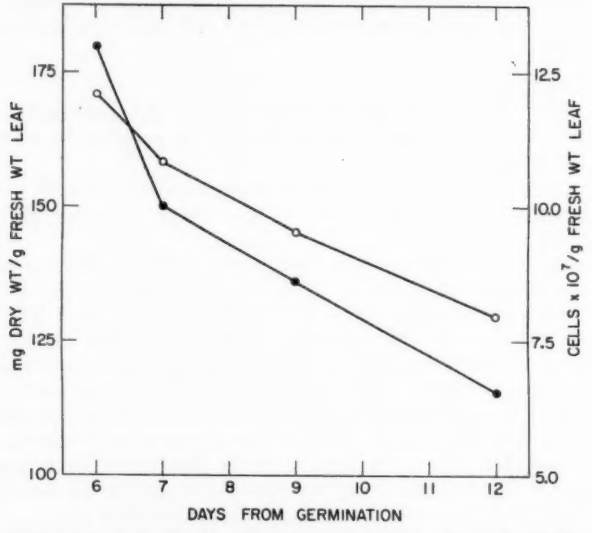


FIG. 2. Dry weight and cell count of pea leaves at various stages of their development. ○ Dry weight per leaf fresh weight. ● Cell number per leaf fresh weight. Each point is the mean of determinations on four individual samples.

The change in leaf fresh weight as a function of growth is depicted in Fig. 3. The fresh weight increased until stage 4, by which time the leaves were fully expanded (cf. Fig. 1). By stage 5 in leaf development the leaf fresh weight had decreased slightly.

*Soluble Protein*

No striking changes were noted in the soluble protein contents of the developing leaves, although there was some decrease after stage 3 (Fig. 3). This was

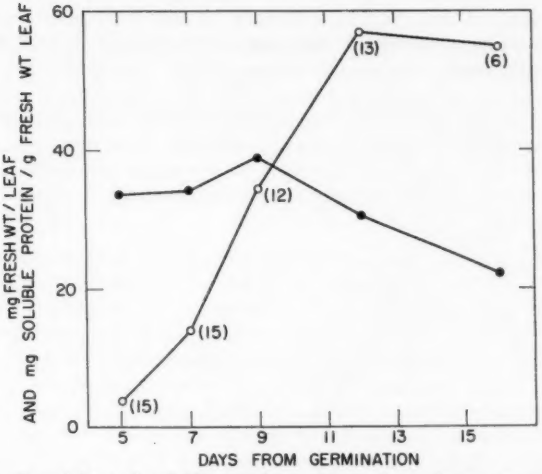


FIG. 3. Fresh weight and soluble protein contents of pea leaves at various stages of their development. ○ Fresh weight per single leaf. The numbers in parenthesis beside each point show the number of individual leaf samples used. ● Soluble protein per leaf fresh weight. Each point represents the mean of determinations on three individual leaf samples.

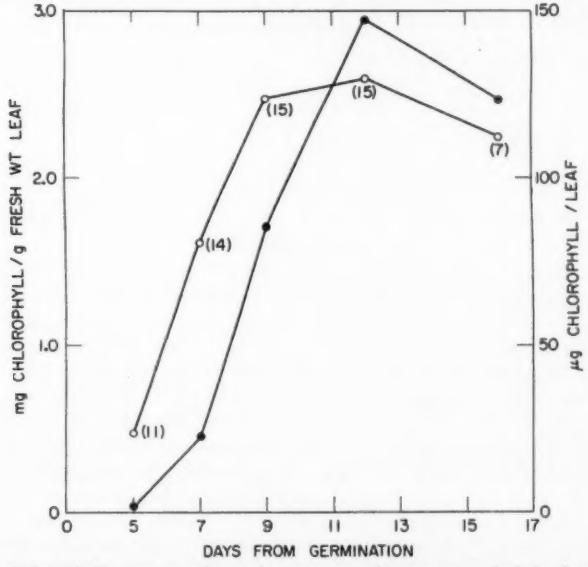


FIG. 4. Chlorophyll content of pea leaves at various stages of their development. ○ Total chlorophyll per leaf fresh weight. The numbers in parenthesis beside each point show the number of individual samples used. ● Total chlorophyll per single leaf. The values were calculated from the chlorophyll per leaf fresh weight and the data in Fig. 3.

consistent with the activities of a number of leaf enzymes, which with few exceptions showed a continued decrease after stage 3 in leaf development (Smillie, unpublished).

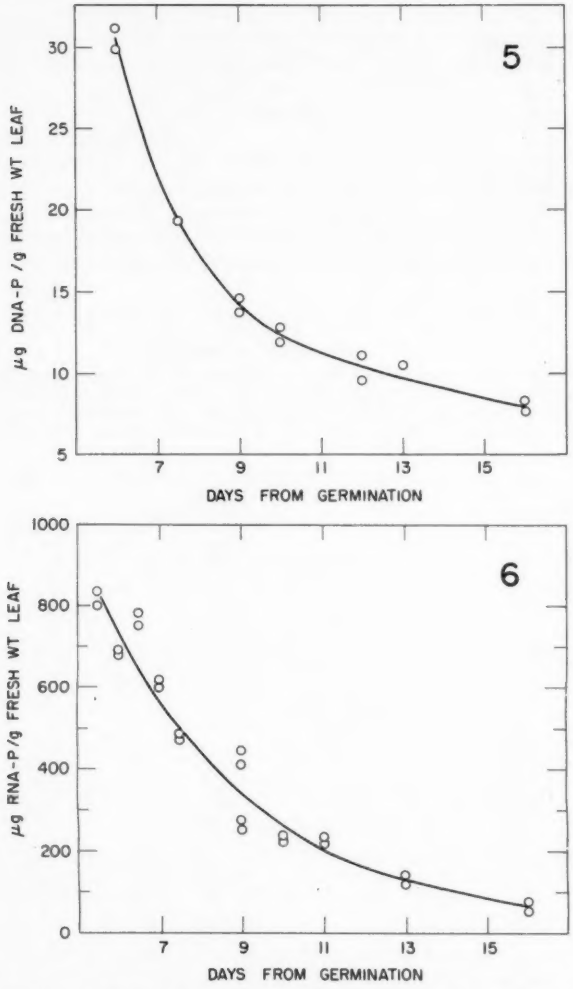


FIG. 5. DNA content of pea leaf homogenates at various stages of leaf development. Each point is an average of duplicate analyses on individual leaf samples.

FIG. 6. RNA content of pea leaf homogenates at various stages of leaf development. Each point is an average of duplicate analyses on individual leaf samples.

*Chlorophyll*

Figure 4 illustrates the changes with age of total chlorophyll per leaf fresh weight and per single leaf. The chlorophyll content was initially low, since the very early development of the immature leaves took place before the plant had emerged from the soil. Although the chlorophyll content expressed on a fresh leaf weight basis was the highest at stage 4, the rapid increase in chlorophyll content had ceased by stage 3. On the other hand, the chlorophyll content per single leaf showed a continuous rapid increase until stage 4.

*Nucleic Acid*

The DNA content of leaf homogenates expressed on a leaf fresh weight basis decreased continuously as the leaves aged (Fig. 5). The decrease was most marked during the early period of leaf expansion. The RNA content of leaf homogenates exhibited a similar although more striking decline as the leaves aged (Fig. 6). These results showed that in contrast to soluble protein or chlorophyll, the highest concentrations of DNA and RNA were found in the youngest leaf samples. By stage 5, the leaf RNA content had fallen to less than 10% of the value found in leaves at stage 1. There was, however, a net increase in RNA per leaf until stage 3 in leaf development. This is evident when the data in Fig. 6 are expressed as RNA per leaf (Table I).

TABLE I

RNA per leaf as a function of leaf age

| Stage of growth | Days from germination | Mg fresh weight of leaf* | μg RNA–P per g fresh weight of leaf† | μg RNA–P per leaf |
|---|---|---|---|---|
| 1 | 6 | 9.0 | 740 | 6.6 |
| 2 | 7 | 13.9 | 570 | 7.9 |
| 3 | 9 | 34.4 | 342 | 11.8 |
| 4 | 12 | 56.9 | 160 | 9.1 |
| 5 | 16 | 54.9 | 60 | 3.3 |

*Calculated from data given in Fig. 3.
†Calculated from data given in Fig. 6.

## Discussion

Most leaves show a general decrease in protein content upon maturation (25, 37). For example, in tobacco (12, 19) and sugar cane (26) the total N per dry weight is greater in young leaves than in old leaves. The changes are often small and little change may occur until the leaves are relatively aged (11). Changes in the soluble protein content of pea leaves as a function of age were not striking (Fig. 3). A small but significant decrease occurred after stage 3 in leaf development. On a dry weight basis, the changes after stage 3 would have been even less pronounced. It is probable that if any marked decrease in protein content did occur, it would have been only evident in leaves older than those used in the experiments.

Although the protein level in growing pea leaves was relatively constant, it cannot be assumed that the enzymic activity of the leaves remained unchanged. The protein contents presumably represented the net change in the levels of enzymes involved in a number of different metabolic pathways.

The relative roles of these pathways in the metabolism of the leaf may have changed as the leaf aged. For instance it was observed that the levels of certain glycolytic enzymes as a function of leaf age were considerably different from a number of enzymes which participated in photosynthesis (33). Reports in the literature indicate an increase in some hydrolytic enzymes during leaf maturation. Also the possible existence of inert or storage proteins in leaves cannot be discounted (24).

A decrease in leaf nucleic acid with age has been noted previously. In tomato leaves the percentage of RNA decreased as the leaves aged (8). Holden (12) showed that the DNA and RNA per dry weight of leaf were considerably higher in young top leaves of the tobacco plant when compared with the large bottom leaves. The larger young leaves were intermediate in their nucleic acid content. Pirie (23) extracted nucleoprotein from immature tobacco leaves, but found much less nucleoprotein in sap from mature leaves although the latter may have been due partially to a lowered extraction efficiency in the case of the mature leaves (24). In some deciduous trees the percentage of nucleic acid remained either fairly constant or exhibited a slight decrease until the onset of fall discoloration at which time the nucleic acid usually decreased (7). Mothes *et al.* (19) noted that during the growth of *Nicotiana rustica* leaves there occurred an initial increase in the leaf nucleic acid followed by a decrease which eventually attained a fairly constant level. In bean (13) and pea (36) roots, it has been found that the percentage of RNA decreased during elongation.

The decrease in the leaf nucleic acid during aging raises the question of the fate of the products of nucleic acid degradation, presumably soluble nucleotides, and related compounds. These may accumulate in the leaves; alternatively, they may be transported to newly forming parts of the plant where resynthesis to nucleic acid may occur. In certain deciduous trees which showed a marked decline in nucleic acid and an increase in acid-soluble phosphorus before defoliation, a back-migration of nucleotides from the leaves has been suggested (7). A similar type of process appears to take place during the germination of certain seeds. Osawa and Oota (21, 22) found that during germination of the bean *Vigna sesquipedalis* a rapid decrease in the nucleic acid content of the cotyledon took place. This was accompanied by a parallel increase in the various parts of the growing seedling, including the plumule. It was suggested that the cotyledons contained storage nucleic acid which was broken down during germination and transported to the growing parts where resynthesis occurred. Judging from their own experiments, Barker and Douglas (2) suggested that similar events occurred in germinating pea seeds.

Few studies have been reported on the nucleotide content of green leaves. The leaves appear to contain a higher percentage of nucleotides, especially adenine nucleotides, than do roots (27) although considerable variation may be expected depending on the age of the tissues investigated. Cherry and Hageman (4) have made an extensive study of nucleotide levels in developing etiolated corn seedlings. In general, the mononucleotides per dry weight increased continuously over the 6-day experimental period. The di- and trinucleotide levels increased for the first 4 days then remained constant for the next 2 days.

The sharp drop in the RNA content of growing pea leaves (Fig. 6) is of interest in considering the possible existence of nucleoproteins in chloroplasts. It has been reported in a number of cases that isolated chloroplasts contained nucleic acid (5, 6, 12, 15, 16, 20, 28, 31), although in some instances the amount found was quite small when compared with the total nucleic acid of the leaf. The fact that in the present study the RNA fell at a time when the chlorophyll was increasing rapidly may indicate that RNA is high only in young plastids. Plastid development involves a period of division and expansion (10, 14) and it is conceivable that like the leaf as a whole, the nucleic acid in the chloroplasts decreases during their maturation. If this is the case it might partially explain some of the discrepancies in the literature regarding the nucleic acid content of chloroplasts. In this connection it is of interest to note that chloroplast fractions isolated from old leaves contained less nucleic acid than those prepared from young leaves (12, 28).

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# PYRENOPHORA PHAEOCOMES (REB. EX FR.) FR.[1]

R. A. SHOEMAKER[2]

## Abstract

The history of *Pyrenophora* Fr. was reviewed with special reference to *Pleospora* Rab. It was concluded that the two genera are distinct. The following characteristics were found to be of diagnostic value for *Pyrenophora*: ascocarps very large, sclerotioid, slow-maturing, usually setose, occurring on grasses; ascospores large, nearly cylindrical with rounded ends, with 3–5–7 transverse septa, and a longitudinal septum in from one to all cells depending on species. In addition, the known conidial states of *Pyrenophora* were noted to be species of the hyphomycete genus *Drechslera* Ito.

An authenticated specimen of *P. phaeocomes* from the Fries Herbarium was used as a neotype in the apparent absence of Rebentisch's specimen. Numerous ascospores were found among the immature ascocarps on the leaves, and taken as evidence that the fungus is an ascomycete. The conidial-state association was tenuously confirmed by the presence of one *Drechslera* conidium. It was concluded that the name *Pyrenophora* can be used for the genus of ascomycetes typified by *P. phaeocomes*.

## Introduction

The name *Pyrenophora* Fr. (Fries 1849, p. 397) is used frequently for some ascomycetes parasitic on cereals and other grasses. The conidial states of these fungi are members of a compact, easily delimited hyphomycete genus, *Drechslera* Ito (1930, p. 355). The relationship between perfect and conidial state can be illustrated by these four examples: *Pyrenophora graminea* Ito & Kurib. in Ito and *Drechslera graminea* (Rab. ex Schlecht.) Shoemaker (1959); *P. teres* Drechs. and *D. teres* (Sacc.) Shoemaker; *P. avenae* Ito & Kurib. in Ito and *D. avenacea* (Curt. ex Cooke) Shoemaker; and *P. bromi* (Died.) Drechs. and *D. bromi* (Died). Shoemaker. If one recognizes the genus *Pyrenophora* it is important to distinguish it from *Pleospora*, not for reasons of nomenclature because *Pyrenophora* is the prior name, but to distinguish it from its nearest ally. Wehmeyer (1953, p. 570) proposed (i) "that until the proper taxonomic limits of the group of species about *Pleospora* [*Pyrenophora*] *phaeocomes* can be determined, they be retained as a sub-group of the genus *Pleospora*." (ii) "that the genus name *Pyrenophora* be removed from the Ascomycetes to the Fungi Imperfecti as a form genus name." The continued use of *Pyrenophora* (for ascomycetes) would be in contradiction to Wehmeyer's conclusion that *Pyrenophora* is not a legitimate name for a genus of ascomycetes. In the rest of this paper are presented the observations that caused me to reach the opposite conclusions: (i) *Pyrenophora* is a well-circumscribed genus distinct from *Pleospora* and (ii) the name *Pyrenophora* is legitimate, and as implied by the title, the correct name of the genus of ascomycetes typified by *Pyrenophora phaeocomes*.

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## Taxonomy

When described, *Pyrenophora* contained three species: *P. phaeocomes* (Reb. ex Fr.) Fr., *P. inclusa* (Schm. & Kunze) Fr., and *P. paradoxa* (Fr.) Fr. According to Wehmeyer (1953) a collection of *P. inclusa* in the Fries Herbarium resembles *Sclerotium bifrons* (Ell. & Ev.) ex Sacc. & Syd., and *P. paradoxa* is not represented by a specimen. *P. phaeocomes* was designated the type (Clements and Shear 1931, p. 276), which is not inconsistent with earlier usage, i.e. Fuckel (1869) and Winter (1885). The subsequent discussion will remain largely with this species.

Rebentisch (1804, p. 338) first described *Sphaeria phaeocomes*, the epithet describing the dark hairs, as follows: "simplex sparsa epiphylla globosa, hemisphaeriis utrinque folii pagina pilis erecto-divergentibus obsitis R. Tab. 1. fig. 4a, 4b. In *Holci mollis* foliis languescentibus. Autumno. Sphaeria atra duriuscula globosa intus albida utraque folii pagina pilis obsita. E longinquo Dematium esse videtur at accuratius adspecta et transversim perscissa ab eo facile dignoscitur." I have not been able to locate Rebentisch's Herbarium or this specimen.

Fries (1823, p. 515) treated the species as *Sphaeria*: "489 *S. phaeocomes* sparsa, aterrima, peritheciis globoso-hemisphaericis subastomis pilis erecto-divergentibus obsitis Rebent. Neom. p. 338 t. 1. f. 4. In utraque folii pagina prominet folium findens, ut Sclerotium fulvum. Intus alba est, contextu Sclerotii, sed in centro adest perithecium minutum nigrum. In foliis exsiccatis graminum vere (v.s.)."

*Pyrenophora* was described as follows (Fries 1849, p. 397): "Nucleus *serotinus*, grumoso-gelatinosus, in stromate ceraceoindurato (Sclerotioideo), perithecii vices gerente, immersus, ostiolo tarde prominule. 'Asci firmi, sporis opacis multiseptatis' Berk. Suppl. VI. t. 11. f. 8. Singulare et affinatis incertae genus, cui plura exotica habemus affinia. Cfr. Dothiorum."

The conception gathered from the generic diagnosis could be briefly stated as: ascocarps consisting of central pseudoparenchyma enclosed by a hard wall, centrum maturing slowly, bearing asci in season, immersed, beak erumpent, appearing late.

*Pyrenophora* as a name conveys the idea of bearing a kernel, seed, or pit within the ascocarp. The generic diagnosis began "Nucleus *serotinus*" referring to the centrum, which matured late. The mature centrum was not described in the generic diagnosis except by reference to Berkeley's (1841) Table XI, Fig. 8. The description of *S. phaeocomes* (Fries 1823) has the pertinent sentence "Intus alba est, contextu Sclerotii, sed in centro adest perithecium minutum nigrum." Fries probably saw the large ascospores, which would appear dark in mass, and interpreted the whole centrum as a perithecium. Winter (1885, p. 522) took the word perithecium too literally and dismissed the entire observation as "nur ein Phantasie-Gebilde". Nevertheless the evidence of the generic name, seed-bearing, the generic diagnosis, nucleus maturing late (but maturation implied), and the description of the centrum of *S. phaeocomes*, small and dark, would substantiate the view that Fries saw mature ascocarps bearing dark ascospores.

The information on asci and ascospores taken from Berkeley's (1841) illustration was of another species, a discordant element that was separated

satisfactorily by Wehmeyer (1953).

The concept of *Pyrenophora* and knowledge of *P. phaeocomes* were rather nebulous until the work of Fuckel (1869). He noted the tendency of *P. phaeocomes* to mature only after overwintering, described asci and ascospores, and produced a figure of one ascospore (Fuckel 1869, Plate VI, Fig. 41). Perhaps most important, he observed a conidial state which he found closely resembled the fungus in the Tulasnes' (1863) Plate 29. The Tulasnes' fungus drawn from material on *Avena* can be readily referred to *Pyrenophora avenae* Ito & Kurib. in Ito by its very characteristic 5-septate ascospores. The conidia are of *Drechslera avenacea* (Curt. ex Cooke) Shoemaker. The important point is that Fuckel found a *Drechslera* conidial state of *P. phaeocomes*.

In his description of *Pyrenophora*, Fuckel stressed the sclerotioid nature of the young ascocarp, the slow development of asci, the hair-like conidiophores on ascocarps, the large size of the ascospores, as well as the presence of conidia. He included in *Pyrenophora* three species: *P. phaeocomes*, *P. relicina* (Fr.) Fuckel, and *P. trichostoma* (Fr.) Fuckel, all on grasses, and one doubtfully included species. In his treatment *Pyrenophora* was not allied with *Pleospora* in the Pleosporeae but included in Dothidiaceae.

The distinctions between *Pyrenophora* and *Pleospora* tended to become obscured as a result of Saccardo's (1883, p. 277 *et seq.*) compilation. He incorporated Fuckel's descriptions of the four *Pyrenophora* species in *Pyrenophora* but as a subgenus, *Eu-Pyrenophora* which was characterized by the features "peritheciis duris, sclerotioideis". The other subgenus of *Pyrenophora*, *Chaetoplea*, was described by "peritheciis coriaceo-membraneis". Subgenus *Chaetoplea* consisted of 26 species with setose ascocarps. Twenty-five of the species were direct transfers from *Pleospora*. The key (Saccardo 1883, p. 238) indicated the distinction between these genera was the presence of setae on ascocarps of *Pyrenophora* and the absence of setae from ascocarps of *Pleospora*. The well-defined limits of *Pyrenophora* as stated in Fuckel's (1869) treatment had thereby been obscured and a single characteristic, the setose nature of the ascocarp, used instead to distinguish *Pyrenophora* from *Pleospora*.

Winter (1885) took exception to the trivial distinction of presence or absence of setae to distinguish two genera and included the species of both genera in *Pleospora*. Wehmeyer (1948, p. 274) concluded that the setose condition seems to be of no more than specific or even varietal or habit rank. Drechsler (1934) in a penetrating but infrequently cited review agreed with Winter that the presence of setae alone was not enough to separate *Pyrenophora* from *Pleospora*. Drechsler noted that *Pyrenophora* antedates *Pleospora*, and drew attention to certain distinctive features of life history and ascocarp structure of *Pyrenophora* that separate it from *Pleospora*. He emphasized the connection between *Pyrenophora* and the conidial state *Drechslera* as found by Fuckel for *P. phaeocomes*. Drechsler (1923) had established the connection between *P. teres* and *D. teres*, *P. tritici-repentis* (Died.) Drechs. and *D. tritici-repentis* (Died.) Shoemaker, and between *P. bromi* and *D. bromi*. At about the same time Ito and Kuribayashi (1931) published an account connecting five species of *Pyrenophora* with *Drechslera* states. Drechsler (1934) noted also that *Pyrenophora* ascocarps were characteristically hard and sclerotioid when young, and ascus development was usually delayed and somewhat protracted.

A summary of Drechsler's (1934) characterization of *Pyrenophora* is: (i) ascocarps hard and sclerotioid when young, (ii) ascus development usually delayed and somewhat protracted, (iii) associated *Drechslera* conidial state.

Wehmeyer's (1949) work on the pattern of development of *Pleospora* ascospores brought out a further distinction in the size and form of *Pyrenophora* ascospores. He treated a number of *Pyrenophora* species as a species complex under the binomial *Pleospora trichostoma*. He found *P. trichostoma* to be unrelated to the three main developmental series of *Pleospora:* leptosphaeroid, vulgaris, and herbarum, because the ascospores were cylindric–ellipsoid with almost straight side walls and rounded ends. He also noted the relatively large size of the ascospores, and his figures (Figs. 1–10, p. 569) make this difference very clear. The ascospores were described as 3-septate with a longitudinal septum in one or both median cells.

The species complex had an extensive synonymy including many species of *Pyrenophora* and was a temporary resting place for the species involved. The inclusion *P. avenae* was questioned (Kenneth 1958, p. 195) because the ascospores are 5-septate. It is doubtful if Wehmeyer saw material of this species because none of the three specimens he cited was on *Avena*, and he described the spores as 3-septate. Nevertheless, the ascospores of *P. avenae* are of the same general form as the other species but quite distinct in septation. *P. phaeocomes* collections studied later by Wehmeyer (1953) had 4–6(7) septa but were also of the same general form. Wehmeyer's reference to and discussion of Fuckel's (1869) Fig. 31 of Plate VI, an appendaged, 3-septate spore of '*Rhopographus filicinus*', was a mistake for Fig. 41, a 7-septate ascospore of *P. phaeocomes*.

Although one may not agree with the treatment of many species as a complex under *Pleospora trichostoma* and the retention of the group of species about *Pyrenophora phaeocomes* as a subgroup of *Pleospora*, it must be admitted that by using ascospore form Wehmeyer brought together a closely related group of species. Ascospore form is included below with other features that have been found to be characteristic of *Pyrenophora*.

In summary, the following criteria seem to characterize *Pyrenophora* and, in particular, distinguish it from its ally *Pleospora*. Ascocarps, very large, hard, sclerotioid when young, wall dark, usually setose and may also bear conidiophores, centrum maturing slowly, usually after overwintering, asci few, large, beak may appear late or be lacking. Ascospores large, light brown, cylindric–ellipsoid with almost straight side walls and broadly rounded ends, 3–5–7 transverse septa, and a single longitudinal septum in from one to all cells, depending on species. Conidial state, *Drechslera* species. Host, Gramineae, often quite specialized to a particular host. This concept is very close to the one published by Müller (1951) except for the inclusion of the *Drechslera* conidial state and grass hosts, which might result in the exclusion of the species on *Typha* and on *Scirpus*.

## Nomenclature

Even if the concept of *Pyrenophora* as a genus distinct from *Pleospora* is accepted, there is an impediment to the use of the name *Pyrenophora*. Wehmeyer (1953) published an account of his study of the only specimen of

PLATE I

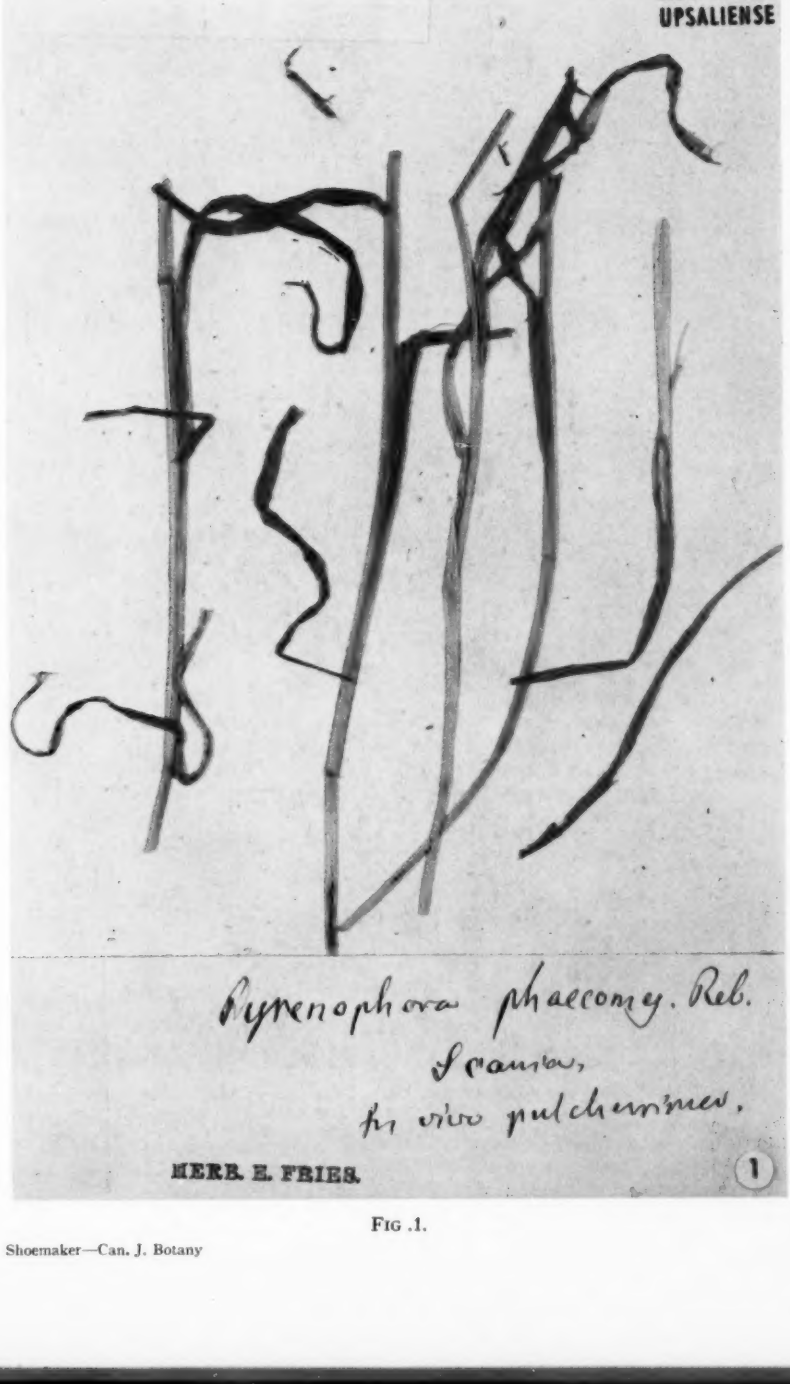
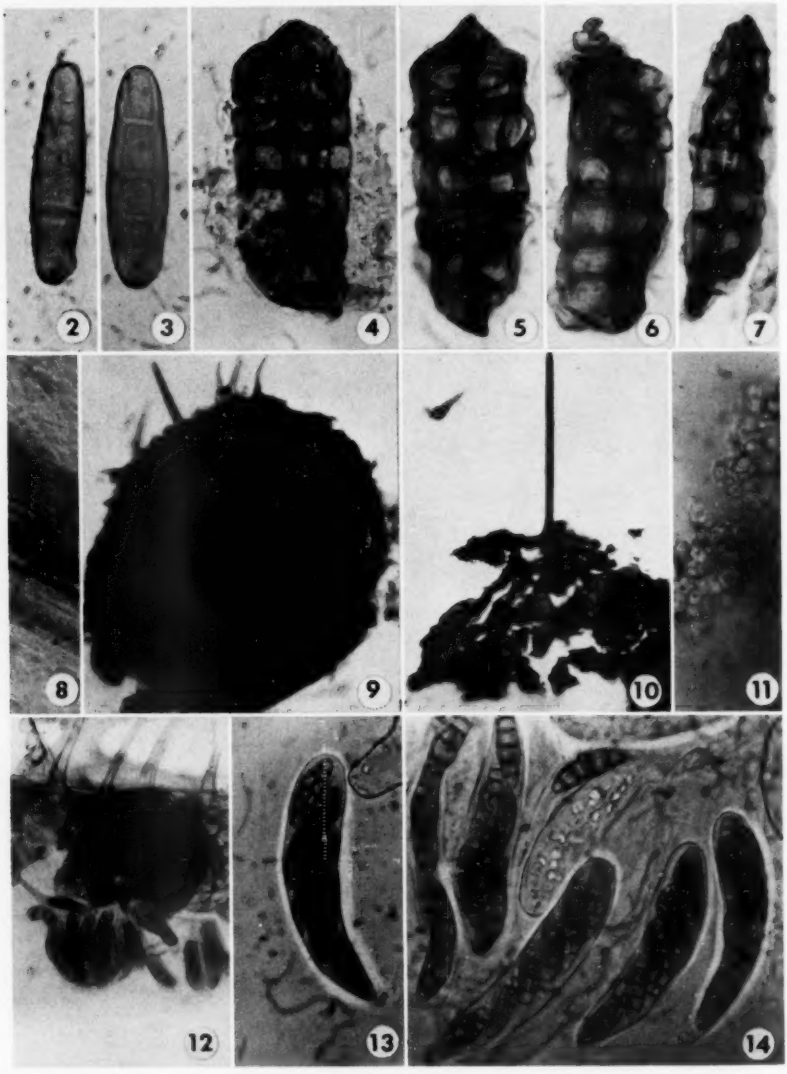

MUS. BOTAN.
UPSALIENSE
Hyphoma phaeocomy. Reb.
Scandia,
in silva pulcherrimus.
HERB. E. FRIES.
1

FIG .1.




FIGS. 2–11. *Pyrenophora phaeocomes* ex Herb. Fries (except Fig. 7). Fig. 2. Conidium in KOH (×500). Fig. 3. Conidium slightly flattened, in glycerine jelly (×500). Figs. 4–7. Ascospores from leaf surface (×500). Fig. 7. Ascospore from Libert, Pl. Crypt. No. 44 in DAOM. Fig. 8. Setose ascocarp (×16). Fig. 9. Ascocarp (×100). Fig. 10. Ascocarp wall (×100). Fig. 11. Pseudoparenchyma (×300). Figs. 12–14. *Pleospora* sp. Fig. 12. Crushed ascocarp (×100). Figs. 13 and 14. Asci and ascospores (×500).

*P. phaeocomes* from the Fries Herbarium. The study was made several years earlier because Wehmeyer's annotation was dated 1/12/1950. He examined 12–15 young ascocarps, found them to be sterile, and concluded that the name could not be used for ascomycetes because the specimen did not bear ascospores. Art. 69 (Lanjouw 1952) stated "The type specimen of a state must bear that state." In the same article was given this explanation: "The perfect state is that which bears asci in the Ascomycetes...." In the 1956 Code (Lanjouw 1956) a different wording was used for the sentence quoted first: "The type specimen of a name applied to a particular state must show the characteristics of that stage."

In the hope of finding asci, ascospores, and conidia, I borrowed the specimen cited by Wehmeyer from the Fries Herbarium. A natural size photograph of this specimen appears as Fig. 1. The label reads: "*Pyrenophora phaeocomes* Reb. Scania, in vivo pulcherrima, Herb. E. Fries." It seems unlikely that this is the specimen Fries (1823) described as "*Sphaeria phaeocomes*...in foliis exsiccatis graminum vere (v.s.)" because the name *Sphaeria* does not occur on the label, and the specimen is labelled in vivo, but Fries noted by v.s. that he saw a dried specimen rather than a living plant. It is authenticated by Fries in his handwriting (see Hughes 1960, Figs. 14, 21, 22) and was used as "lectotype" by Wehmeyer (1953). If Rebentisch's specimen or other more pertinent material Fries studied is found, the choice of the type specimen would need to be reconsidered.

The leaves were heavily infected with young ascocarps, appeared to have been necrotic when collected, but were not overgrown by saprophytic fungi. Two leaf tips did have some molds present but the main specimen was quite uniformly infected by *P. phaeocomes*. The young ascocarps were dark brown, spherical, partly erumpent from the thin leaves, without a beak or an ostiolar opening, but furnished with numerous setae at the apex. Three ascocarps were examined. They consisted of a large, white central mass of undifferentiated pseudoparenchyma surrounded by a thin, dark brown wall (Figs. 10, 11). No evidence of even young asci was observed in these preparations.

Ascospores were found on the leaf surface. These ascospores were very large 82–97×30–37 $\mu$, nearly cylindrical, with a median septum in all cells and (5)6 transverse septa (Figs. 4, 5, 6). The spores were swollen, devoid of protoplasts, medium brown, and surrounded by the remnants of the gelatinous sheath. No clear evidence of germ tube production was seen, but the empty, swollen cells resembled those of germinated ascospores. I think the ascospores were the original inoculum that produced the infection which had developed only to the incipient ascocarp or protothecium (Shoemaker 1955) stage when the collection was made. No date is on the specimen but the phrase in vivo, the condition of the leaves, and the lack of saprophytes on the leaves all indicate that the specimen had not overwintered.

Fries's specimen was quite similar to the DAOM specimen of Libert's Plantae Cryptogamicae Arduennae. 1830. 44. *Sphaeria phaeocomes*, Rebent. Neom. p. 338.—Fr. Syst. Myc. 2. p. 515. In foliis exsiccatis *Holci mollis*. Autumno. In the latter specimen ascocarps were immature with only pseudoparenchyma in the center. Ascospores were detected on the leaf, usually on a bleached, whitish zone in the center of a lesion. The ascospores (Fig. 7) were

shrunken and empty as though germinated. Some conidiophores and one distorted *Drechslera* conidium were found. The similar condition of the leaves and the similar vegetative characteristics of the grass hosts of the two collections indicated that Fries's specimen was also an autumn collection and probably on the same grass, *Holcus mollis*.

Fuckel (1869) and Winter (1885) both mentioned the importance of overwintering for the maturation of asci in *P. phaeocomes*. Wehmeyer (1953) found all 12–15 ascocarps examined from the neotype to be sterile, which is typical of autumn collections. I did not find even young asci in the three ascocarps examined. The evidence supports the view that the ascospores on the leaf were initial inoculum and the ascocarps were immature when collected. The presence of the characteristic ascospores and the reasonably distinctive ascocarps on the neotype is enough to permit identification of later collections with this ascomycete.

Additional evidence on the identity of the fungus came from one conidium taken from the leaf surface directly below the setae on the ascocarp shown in Fig. 8. The conidium (Figs. 2, 3) was light yellowish brown except for the lighter basal cell, which had a dark scar 6 $\mu$ wide included in the contour; subcylindric, widest point 0.45, tapered nearly equally to each end; apex a broad hemiellipse; basal cell distinctive, contour nearly parallel on sides and base nearly truncate; septa thin and obscure when revived in potassium hydroxide (Fig. 2), broad and distinct in glycerine jelly (Fig. 3); cells subequal, shorter than wide, $\mp$13.2 $\mu$ long; germ tube emerging laterally from basal cell; 66 $\times$ 18 $\mu$. This one conidium shows all the characteristics of *Drechslera*. A conidial state for *P. phaeocomes* has been noted (Fuckel 1869, Graham 1955) but not named. The presence of the conidium gives confirmation of the *Pyrenophora–Drechslera* association.

The host plant usually gives a clue to the identity of the fungus. Rebentisch (1804), Greville (1824), Fuckel (1869), and Winter (1885) recorded it on *Holcus mollis*. Müller (1951) reported it on *Holcus Lanatus* and *Calamagrostis varia*. Wehmeyer (1953) studied a specimen collected by Lennart Holm on *Anthoxanthum odoratum* from Sweden, and Graham (1955) reported the fungus in North America on *Dactylis glomerata*. The usual host is *Holcus*. Fries's specimen has only leaves and culms present and the material resembles the Libert collection of *Holcus mollis* but a precise identification is not possible. In view of the fairly wide host range reported it may be enough to know that it occurred "in foliis exsiccatis graminum vere".

It is of interest, but not of particular consequence, that the tip of one leaf is overgrown by mold and bears some small, glabrous, thin-walled, beaked, light brown ascocarps (Fig. 12) which contain a central fascicle of numerous, narrow asci bearing 5-septate, elliptical ascospores of a *Pleospora* (Figs. 13, 14). This material is mentioned only to prevent confusion in the future, should someone examine this specimen and find my slides of this *Pleospora*, or locate more of the small glabrous ascocarps on the specimen.

The nomenclatural problem arising from the nature of the type specimen of *P. phaeocomes* cannot be considered completely solved. The argument centers on the interpretation of Art. 59 (Lanjouw 1956). It seems to me that the principle that a type of a name applied to a perfect state must show the

characteristics of that state has been met. The characteristics (of prime importance for this genus and species) are shown by the specimen: the large, sclerotioid, setose, globose ascocarps with a pseudoparenchymatous centrum and a very dark brown wall, erumpent from grass leaves, and with large subcylindric ascospores with one vertical septum through all six or seven cells. Asci were not seen but they are not of much diagnostic value in distinguishing species in this group. The part of Art. 59: "The perfect state is that which bears asci in the Ascomycetes. . ." seems to me to be explanation of the term perfect state rather than a precise definition of what characteristics shall necessarily be present.

*Pyrenophora* has characteristics of ascocarp form and development, ascospore form, and host range that permit its recognition as a genus that is distinct from *Pleospora*. The evidence of the association between *Pyrenophora* and the hyphomycete genus *Drechslera* supports the concept of *Pyrenophora* as a small, compact genus. The neotype of the type species, *P. phaeocomes*, shows the characteristics of this ascomycete in sufficient detail to permit the identification of later collections of this species, and the recognition of the features that ally other members of the genus with it: ascocarp morphology and development, ascospore form and size, *Drechslera* conidial state, and grass host. Although the genus has been treated at subgeneric levels by authors who stressed particular characteristics the constitution of the taxon has remained quite stable and its recognition does not involve major nomenclatural changes. The legitimacy of *Pyrenophora* was questioned because of the apparent sterile nature of the neotype of *P. phaeocomes* but a re-examination of the specimen revealed ascospores characteristic of *P. phaeocomes* and a conidium of its *Drechslera* conidial state. Although the evidence from the neotype was meager, and required interpretation in relation to later collections it is concluded that *Pyrenophora* is a legitimate name, and the correct name of the ascomycete genus typified by *Pyrenophora phaeocomes*.

## Acknowledgments

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# THE BOTANY OF THE NORTHWESTERN QUEEN ELIZABETH ISLANDS[1]

D. B. O. SAVILE


## Abstract

Ellef Ringnes Island has a confirmed flora of 49 vascular plants and five parasitic fungi. The adjacent islands have less diversity of habitat and probably have even poorer floras. There are no endemics and the plants are extremely depauperate. The summer climate at Isachsen is colder than at any other station in the Canadian arctic. Although there are no convincing indications that Ellef Ringnes I. was overrun by a Wisconsin continental ice sheet, it cannot have escaped being snow-covered. The light cover of snow and ice on the outer islands was quickly lost in the postglacial xerothermic, which enabled plants to spread along the periphery of the archipelago. The numerous plants that occur southwest and northeast of these islands but not in them indicate that postglacial cold periods, probably accompanied by at least partial snow cover of the outermost islands, have driven out many species. Nearctic refugia are discussed and it is indicated, by analysis of distribution patterns, that no refugia occurred in the Canadian arctic archipelago. The region has been colonized from the Peary Land refuge, the Yukon–Alaska refugia, and from south of the retreating ice sheets.


## Introduction

Ellef Ringnes Island, discovered only in 1901, was until recently extremely inaccessible. Consequently no significant biological work was attempted there until the last decade. The island is within a substantial area in which no definite sign of Wisconsin glaciation has been found and which is indicated as unglaciated on the Glacial Map of Canada (Geological Association of Canada, 1958). No clear indications of glaciation have been reported on substantial parts of Prince Patrick, Melville, and Bathurst Islands or on the whole of Ellef Ringnes, Amund Ringnes, Cornwall, King Christian, Lougheed, Borden, Mackenzie King, Brock, and some of the small islands adjacent to Melville and Bathurst. The need for detailed botanical studies within this area is clear. I accordingly welcomed the opportunity to become attached during the summer of 1960 to the Polar Continental Shelf Project with headquarters at Isachsen, the site of the joint arctic weather station on Ellef Ringes I. I was at Isachsen from June 15 to August 12. Most of my work was done within walking distance of Isachsen; but 2 days were spent at a camp on the Christopher Peninsula, and brief visits were made to Lougheed I., Meighen I., northern Borden I., Cape Isachsen, and a sandstone area 10 miles from Isachsen.

Lichens, bryophytes, all groups of fungi, and vascular plants were collected. The lichens and bryophytes are being studied by Dr. W. A. Weber and Dr. W. C. Steere respectively. This paper includes discussions of the vascular plants and parasitic fungi. The saprophytic fungi, which scarcely affect the

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phytogeographic picture and which pose several taxonomic problems, will not be treated here.

The Queen Elizabeth Islands, comprising all the islands of the Canadian Arctic Archipelago north of the natural division formed by M'Clure Strait, Viscount Melville Sound, Barrow Strait, and Lancaster Sound, have been variously subdivided. The southern string of islands from Prince Patrick to Devon are segregated as the Parry Islands. Axel Heiberg and the Ringnes Islands, discovered during the second voyage of the *Fram*, are sometimes designated the Sverdrup Islands. However, the Ringnes Islands are much more closely related, physiographically, climatically, and biologically, to the small islands adjoining them than they are to mountainous Axel Heiberg. For lack of a formal name to segregate them, I am using the term northwestern Queen Elizabeth Islands for this group of small outer islands: Meighen, Ellef Ringnes, Amund Ringnes, King Christian, Lougheed, Borden, Mackenzie King, and Brock. The positions of these islands are shown on Fig. 1.

## Historical Background

Amund Ringnes I. was sighted in 1900 by Sverdrup, during the second voyage of the *Fram*. Isachsen and Hassel were detached to make a brief reconnaissance of its northeast coast, but they lacked supplies for more extensive work. On April 8, 1901, Isachsen and Hassel left the *Fram*, in Goose Fiord, and made a sled trip round both Ellef and Amund Ringnes Is., during which they also sighted King Christian I. They returned to the *Fram* on June 12. Simmons (1913) credits them with collecting six species of plants, which will be discussed below under Vascular Plants. Such specimens would inevitably have been overwintered plants. The Ringnes Is. were not revisited until 1916. In April of that year MacMillan visited King Christian I. and the south coasts of Ellef and Amund Ringnes. In the same year Stefansson travelled north past Brock and Borden Is., rounded Cape Isachsen on northwestern Ellef Ringnes, and continued northward to discover Meighen I. on June 15. He then turned south and passed through Hassel Sd., which separates the two Ringnes Is. The next year, after travelling over the sea to the northwest, Stefansson mapped the coast along Deer Bay where the Isachsen weather station is now situated. Ellef Ringnes was next visited in 1929 when, in the course of a notable R.C.M.P. patrol, Sgt. Joy touched the south coast. It then remained unvisited until the weather station was set up in 1948. Taylor (1955) gives further details and a bibliography of the exploration of these islands.

The botanical history of our region may be said to date from 1948 when Innis Taylor collected specimens of seven species of plants at Isachsen between July 16 and 30. Heywood (1957) studied the geology of the Isachsen area in 1952 and 1953, and collected 16 species of plants mainly in 1952. MacDonald (1961) made a biological survey of the Isachsen and Louise Fiord areas in 1954. He made extensive collections of flowering plants at Isachsen, recording 35 species. R. Thorsteinsson collected a few plants on Meighen I. in 1957 and on Mackenzie King I. in 1958 during geological investigations. All these specimens are in the National Museum.

## General Description

Ellef Ringnes I. lies between 77°45′ to 79°20′ N. and 99° to 106° W.; and, with an area of 5139 square miles, is substantially the largest of the northwestern Queen Elizabeth Is. It also has the highest relief and greatest variety of habitats of this group of islands. It is accordingly probable that it has the largest flora of the group. Its only possible competitor in this respect is Amund Ringnes, which may harbor a few plants not found on the other islands, because it is closer to Axel Heiberg and somewhat removed from the unbroken sweep of wind from the Arctic Ocean.

In several parts of Ellef Ringnes I. intrusive igneous rock produces steep hills and cliffs up to 800 ft high. Springy areas on and below the talus slopes of these cliffs are among the most fertile habitats and allow a modest diversity of plants that cannot be expected elsewhere. The Isachsen area, fully described by Heywood (1957), is conspicuously rich in these outcrops of basalt, gabbro, and, particularly, diabase. There is thus reason to believe that nearly all the plants that grow on the outer islands occur at Isachsen.

Away from the igneous outcrops the relief is lower. There are some outcrops of shale and sandstone, usually on arid hilltops and almost devoid of plant life; but shallowly rolling plains, of sand and clay in varying proportions and dissected by gulleys, predominate. Such areas probably seldom bear more than 25 species, compared with 48 species taken at Isachsen. Twenty-four species were actually taken on one such area on the Christopher Peninsula in 2 days. This total is probably not quite complete, but it includes several species that probably owed their presence to the proximity of igneous hills.

The northwesternmost part of Ellef Ringnes consists of low-lying sand and gravel of the Beaufort formation. This interesting formation, discussed by Tozer (1960), is apparently very late Tertiary in age. It contains much coniferous wood that is unfossilized but extremely well preserved. The wood is decorticated and superficially weathered but cuts cleanly and is of normal color beneath the surface. Some branches show distinct larval galleries of bark beetles. The three Beaufort formation sites visited proved very sterile. Near the coast it may be almost impossible to find any plants at all. There seems to be an almost complete lack of organic material and microorganisms. Every specimen of *Saxifraga oppositifolia* that I have examined from other arctic stations has yielded one or more species of saprophytic fungi in some abundance, including my Isachsen specimens, which have yielded a total of 10 species. A specimen of this plant from the Beaufort sand of northern Borden I., however, yielded no trace of any fungi. Here the dead plants seem to be broken up by sand abrasion and the pieces washed or blown into the streams. Thus the sand is exceptionally clean. The preservation of the coniferous wood must be due to this lack of any organic substrate for microorganisms, coupled with the fact that the formation has been largely immersed during the interglacials and frozen during the glaciations. In a brief visit to the Decca Navigation Station at the tip of Cape Isachsen I was able to find only very stunted vegetative *Saxifraga cernua*, sterile *Phippsia* sp., and scraps of moss.

Meighen I., which I visited briefly, is situated wholly within the Beaufort

formation and seems to have a very limited flora. At the Decca Navigation Station on the west coast I could find only a trace of *Phippsia concinna* and rare scraps of moss and lichen. A shallow icecap covers about a tenth of the island, so that no part of the island is far from sea or land ice. T. Thorsteinsson collected stunted scraps of *Luzula confusa*, *Papaver radicatum*, *Draba oblongata*, and *Saxifraga oppositifolia* on the island, and it is unlikely that the vascular flora exceeds 20 species.

During a brief stop at the northern tip of Borden I., also low-lying Beaufort sand, not a single plant of any kind could be seen. However, Mr. D. R. Horn, who was working on the island in late July, collected six species of vascular plants somewhat farther inland (*Phippsia algida*, *Stellaria ?crassipes*, *Cerastium arcticum*, *Papaver radicatum*, *Saxifraga cernua*, and *S. oppositifolia*). All except *P. radicatum* were predominantly or completely vegetative.

Lougheed I. was visited on June 19. No new growth of any plant was seen. The shallow, rolling slopes of sand and mud showed abundant old stems of *Alopecurus alpinus* and appreciable *Saxifraga oppositifolia*. To judge from similar habitats on Ellef Ringnes, the total flora of this island must be very small.

In 1958 R. Thorsteinsson collected 13 species of plants on Mackenzie King I.: *Alopecurus alpinus*, *Luzula confusa*, *L. nivalis*, *Cerastium arcticum*, *Ranunculus sulphureus*, *Papaver radicatum*, *Draba bellii*, *D. subcapitata*, *Saxifraga caespitosa*, *S. cernua*, *S. nivalis*, *S. oppositifolia*, and *Potentilla hyparctica*.

## Soil Salinity

A feature that became increasingly clear at Isachsen, as the surface of the mud dried in late June, was that the runoff must be very superficial except on coarse talus slopes; for a crust of salts appeared on all level clay surfaces and on many gentle slopes. Even on gravelly hills the salts are abundant below the surface, for a crust appeared on a hill at 250 ft where gravel had been removed for construction work. On some of these alkali flats the only plant present was *Puccinellia angustata*, which grew in small clumps spaced at about 18 to 24 inches. Where a mud slide exposed a new surface the crystals grew out as a "fur" about one-quarter inch long.

During the spring runoff the ground is frozen to the surface until almost every scrap of snow has melted. Consequently there is little leaching at this time except on lower slopes or in gulleys. The summer rainfall is often too light to cause much runoff. On substantially level ground such rains as are capable of soaking several inches into the ground are stopped by the permafrost and evaporate with little lateral movement.

## Summer Climate and Weather

### *Comparison with Other Canadian High-Arctic Stations*

Almost every botanical specimen seen from Ellef Ringnes and adjacent islands is exceptionally depauperate, much more so than those from, e.g., Mould Bay, Eureka, and Alert. They are fully as stunted as those from Ward Hunt I. (83°05′ N.) off the north coast of Ellesmere I.

Table I shows the mean summer temperatures of five high-arctic stations.

TABLE I

Summer temperatures at northern arctic stations

| Station | Lat. N. | Long. W. | Mean temp. (°F) | | |
|---|---|---|---|---|---|
| | | | June | July | August |
| Alert | 82° 30′ | 62° 20′ | 31.6 | 39.3 | 33.5 |
| Eureka | 80 00 | 85 56 | 36.9 | 41.9 | 37.8 |
| Isachsen | 78 47 | 103 32 | 31.3 | 38.4 | 34.3 |
| Mould Bay | 76 14 | 119 20 | 31.2 | 38.0 | 34.0 |
| Resolute | 74 43 | 94 59 | 32.6 | 39.7 | 36.8 |

TABLE II

Summer temperature and precipitation at Isachsen

| | Mean temp. (°F) | | | Precipitation (in.) | | |
|---|---|---|---|---|---|---|
| | June | July | August | June | July | August |
| 1954 | 32.0 | 38.0 | 36.8 | .10 | 1.34 | 1.49 |
| 1959 | 32.0 | 42.75 | 35.75 | .12 | .11 | .17 |
| 1960 | 33.35 | 38.15 | 37.45 | .13 | 1.17 | .54 |
| 13-year av. | 31.3 | 38.4 | 34.3 | .14 | .84 | .81 |

The means for Isachsen are corrected up to 1960 from 13 years' records; those from the other stations are from data supplied by the Meteorological Branch up to 1957 and based on 8 to 11 years' records. Although such short term means are not completely reliable for very small differences, it seems clear that Isachsen is cooler than Eureka and Resolute. It is probably slightly cooler than Alert in July, but differs little from Mould Bay. The exceptionally warm July of 1959 at Isachsen puts the mean for that month slightly above that for Mould Bay, but the difference is probably not reliable.

Two conclusions may be drawn from these figures and the botanical evidence. First, when summer mean temperatures are so low a very small temperature difference may have a profound effect on the stature and diversity of the plants. Second, it is clear that air temperature, as measured in the standard thermometer screen, is far from being the sole limiting factor in plant growth. What is actually most important is the temperature for a few centimeters above and below the ground surface. This temperature depends largely on direct insolation and wind velocity. Sunshine data are not kept for any of these stations except Resolute. However, the Meteorological Branch (1959) has issued data on percentage cloud cover at these stations, based on 3–4 years' records. These data indicate that Alert and Eureka have substantially less cloud cover than Isachsen, Mould Bay, and Resolute. The last three stations seem, from the figures, to be about equally cloudy with ca. 80% cloud cover; however, estimation of cloud cover is difficult and somewhat subjective, and small recorded differences may be misleading. The wind data, calculated from 7 years' records, indicate that Mould Bay has a slightly higher wind velocity than Isachsen (12.4 compared with 11.0 m.p.h.) for the summer months of June, July, and August. It thus appears that Isachsen may

suffer, relatively to Mould Bay, mainly through its lower noon solar elevation. Alert and Eureka, although farther north, gain in increased insolation through lower percentage cloud cover, and also have higher air temperatures because of their situation in mountainous country. Most of the air masses reaching them must cross high mountains on which they deposit most of their contained moisture. The latent heat of condensation and freezing is transferred to the air, which, on being dynamically warmed on its descent to the valleys, has a temperature substantially above that at which it left sea level.

The effect of solar elevation on soil temperature is clearly shown in soil temperature charts secured at Isachsen in 1960 by Mr. D. St-Onge, Geographical Branch. On heavily overcast days with rain or snow the amplitude of the diurnal fluctuation at the soil surface was equal to, or even less than, that of the air temperature, e.g. 30–36° F (soil) and 26–37° F (air) on July 20. On predominantly sunny days, however, the soil surface temperature occasionally fluctuated more than 40° F, e.g. 26–72° F (soil) and 27–43° F (air) on June 24. The conspicuous drop in soil temperature at night, even with continuous sunshine, indicates the ineffectiveness of the sun in warming the soil when it is at extremely low elevations.

In 1960 the cloud cover was often not only continuous but very heavy, consisting of several layers. On such occasions the light intensity is low and isolational warming of the soil is meager even at midday.

*Wind Direction and Residual Snowdrifts*

Although level ground at Isachsen was about 50% bare on June 15 and completely bare on June 30, extensive drifts still persisted in gulleys and on lee slopes. Some of these drifts are permanent and are still large in August. The strongest and most persistent winds between September and May, during which drifting may be expected, are from the north and northwest, and many of the larger drifts face south or southeast as expected; but there are enough strong winds from other quarters to produce substantial drifts on various exposures. The largest drifts studied were to the south of cols that provided a natural venturi with a north to south passage. In the throat of such a venturi the wind is evidently often very strong, removing not only snow but soil and gravel so that little remains but boulders.

These residual drifts are important because water seeps from them all summer, providing habitats for plants that would not otherwise become established. The smaller drifts supply mesophytic species that otherwise occur only in small patches on hillsides and talus slopes. Only rarely do true marshes develop. Two occurred below adjacent cols southwest of the station. Two smaller ones were seen below the talus of a high hill to the northwest, and another very small one was found in a springy pocket on the west side of a ridge. All these marshes were associated with extensive drifts. The effect of the residual drifts at Isachsen was somewhat different from that recorded on Somerset I. (Savile, 1959). There the precipitation was heavier, and the residual drifts reduced plant growth by shortening the season. On Ellef Ringnes, exposures without residual drifts are generally very arid. Because some of the cliffs are high and snowfall light, the drifts tend to occur well

up the talus slopes; and the lower talus is exposed early but supplied with enough moisture from the drift to support considerable vegetation.

In June, July, and August the average wind speed is about 11 m.p.h., calms are rare and winds of 30 m.p.h. not uncommon. The prevailing directions are again north and northwest; but other directions are well represented and strong winds may blow from any quarter.

*Comparison of Growing Seasons*

Observations of the 1959 stems of many plants indicated that they were substantially higher than those produced in 1960, except in very arid habitats. Table II shows that July 1959 was exceptionally warm. There was no severe cold in the month, the minimum being 31° F. In contrast, July 1960 was cool and the temperature twice fell to 26°. August 1960 was relatively warm, but the warm weather was virtually confined to the third week, by which time growth had stopped. Growth in 1954 was fully as good as in 1960 and probably slightly better, to judge from MacDonald's specimens. It will be seen that the summer of 1954 closely resembled that of 1960. July was cool and wet in each year, but in 1954 the minimum for the month was 29° F compared with 26° in 1960, and no measurable snow fell in contrast to 5.1 inches in 1960. The first 2 weeks of August 1960 were just as cold and wet as those of August 1954; it is only the warm and dry third week that makes August 1960 appear to be a favorable month.

The less extensive collections of Taylor in 1948 and Heywood in 1952 suggest that growth in these seasons was about as in 1960. It thus seems that 1960 was a nearly typical growing season, a conclusion which is supported by the climatic data. The good growth in 1959 of plants in moist situations was clearly due to the warm, dry weather in July; but plants in arid situations were stunted by lack of moisture. In 1960 plants on dry sites grew moderately

TABLE III

Phenological data for Isachsen

| Species | Date of first flowering | |
|---|---|---|
| | 1954 | 1960 |
| *Saxifraga oppositifolia* | 20 June | 18 June |
| *Ranunculus sabinei* | 25 June | 25 June |
| *Draba oblongata* | 26 June | 24 June |
| *Draba subcapitata* | 26 June | 24 June |
| *Cerastium arcticum* | 26 June | 29 June |
| *Papaver radicatum* | 26 June | 29 June |
| *Saxifraga caespitosa* | | (26) 29 June |
| *Potentilla hyparctica* | | 29 June |
| *Saxifraga rivularis* | | 1 July |
| *Arenaria rubella* | | 4 July |
| *Saxifraga nivalis* | | 4 July |
| *S. flagellaris platysepala* | | 4 July |
| *Eriophorum scheuchzeri* | | 6 July |
| *Alopecurus alpinus* | | 15 July |
| *Festuca brachyphylla* | | 15 July |
| *Pleuropogon sabinei* | | 15 July |
| *Puccinellia angustata* | | 17 July |
| *Dupontia fisheri* | | 28 July |

well, but plants in the marshes made poor growth because the marshes were flooded with cold water for almost the whole growing season. The phenological data presented in Table III allow some comparison of the seasons of 1954 and 1960. Comparison with the data of Bruggemann and Calder (1953) shows that Isachsen compares unfavorably with Alert early in the season.

*Summer Climate of Peary Land*

Because that region must be considered later under Glacial History and Phytogeography, it is worth while to compare the summer climate of Peary Land in northeast Greenland, which ranges from about 82° to 83°35′ N., with that of some of the stations in the Canadian Arctic Archipelago. Fristrup (1952) has published full weather data for 2 years for a Peary Land station. The very close agreement between the two summers suggests that his figures are truly representative of the region. The mean temperatures for June, July, and August are 36.7, 43.4, and 38.5° F, about 5° above the Isachsen means and even slightly above the Isachsen mean daily maxima for these months. Although the August mean is exceeded by Arctic Bay (73°00′ N.) and Pond Inlet (72°43′ N.), the *northernmost Canadian station to exceed the Peary Land means for all 3 months is Holman I.* (70°30′ N.). The relatively southern Canadian stations must have higher soil temperatures, because higher noon solar elevations provide better insolational warming. It must also be remembered that the Canadian stations are all essentially coastal; and that substantially higher summer temperatures would be recorded in the interiors of e.g. Banks and Victoria Is. Nevertheless the contrast in recorded air temperatures is striking, and is vital to the development of a realistic picture of phytogeographic history in the archipelago. Such a picture is, indeed, only now becoming possible with the recent development of an adequate network of stations throughout the archipelago.

The high temperatures of Peary Land seem to be largely due to prevailing winds off the Greenland icecap, which undergo strong dynamic warming. The high temperature is combined with plentiful sunshine and with precipitation that is very low even by arctic standards. This relatively benign summer is reflected in a flora of 97 species of vascular plants, recorded by Holmen (1957). This is almost exactly the size of the flora of Somerset I., nearly 10° further south, and of the combined floras of Ellef Ringnes, Prince Patrick, and Cornwallis Is. This substantial flora is strong evidence that the admittedly short climatic record for Peary Land is realistic even if not extremely accurate. Coupled with the physical evidence and the botanical evidence presented in the final section of this paper, it strongly supports the contention that Peary Land was not fully glaciated in the Wisconsin.

There is one part of the Canadian Arctic Archipelago at the latitude of Peary Land with a comparable flora. This is Hazen Lake, from which ca. 110 species of vascular plants are recorded (J. H. Soper, in lit.). However, Hazen Lake was quite definitely overwhelmed by Wisconsin ice (R. L. Christie, personal communication), and owes its flora to its benign postglacial summers. It is completely ringed by high mountains, except for its narrow outlet valley, and consequently every wind that reaches it is dynamically warmed.

## Birds and Mammals

The vertebrate population of the Isachsen area has been described by MacDonald (1961) and some additional notes given by Savile (1961). The population density is exceptionally low even by arctic standards. The breeding bird density at Isachsen in 1960 was about one-fortieth of the average of estimates for tundra in the low arctic.

The Collared Lemming (*Dicrostonyx groenlandicus*) is of great ecological importance, notably in providing localized nitrification. The only other animal of noticeable importance in this respect is the Brant Goose (*Branta bernicla*), which causes some nitrification in marshes and on springy slopes. The Brant is also probably of major importance in the transport of seeds, as will be discussed in the final section.

## Description of Principal Habitats

In the high arctic, where, as indicated elsewhere (Savile 1960), severity of the physical environment overshadows interspecific competition, no well-defined plant communities occur. A picture of the ecology is accordingly best presented through a description of the chief habitats.

*Rocky Hilltops and Dry Talus Slopes*

These arid areas are virtually unvegetated except for crustose lichens and the conspicuous silvery moss *Rhacomitrium lanuginosum*. In slightly moister depressions *Saxifraga oppositifolia* occasionally occurs but other vascular plants are rare. These areas are abundant near Isachsen.

*Moist Rock and Gravel Slopes below Hills*

Springy strips on the lower talus slopes at Isachsen accumulated cushions of moss in which *Poa arctica* ssp. *caespitans*, *Luzula nivalis*, *Stellaria ciliatosepala*, *Papaver radicatum*, *Saxifraga caespitosa*, and *Potentilla hyparctica* flourished. As these strips become increasingly nitrified by the Collared Lemming, *Poa* seems to increase to some extent.

On muddier slopes *Poa abbreviata*, *Festuca brachyphylla*, *Oxyria digyna*, *Ranunculus nivalis*, *R. sulphureus*, *Draba* spp., *Saxifraga nivalis*, *S. tenuis*, and *S. flagellaris* often occur. Gravel slopes that dry out during the summer are dominated by *Saxifraga oppositifolia*.

A springy gravel slope at 450 ft, just below the summit of a diabase hill on the Christopher Pen. (79°00′ N. 101°38′ W.), was visited on July 8. Almost no new growth had occurred, but scattered and very stunted plants of *Phippsia* sp. (sterile), *Luzula nivalis*, *Papaver radicatum*, *Cardamine bellidifolia*, *Draba bellii*, and *Saxifraga foliolosa* were found. The situation was strikingly similar to the moist slope visited on the high central plateau of Somerset I. (Savile, 1959). If the *Phippsia* seen was *P. algida*, 5 of the 6 species recorded are on the list of 11 species found on the Somerset plateau at 1100 to 1200 ft.

*Clay Plains*

Clay plains form the most extensive habitat at Isachsen. Where they are practically level they are so alkaline that they may bear only *Puccinellia angustata*. With increasing gradients *Alopecurus alpinus*, *Phippsia concinna*, *Cerastium arcticum*, *Ranunculus sabinei*, *Papaver radicatum*, *Draba oblongata*,

*D. subcapitata*, *Saxifraga nivalis*, *S. cernua*, and *Potentilla hyparctica* may be plentiful. On muddy flats below snowbanks or near streams *Cochlearia officinalis*, *Saxifraga rivularis*, and *S. foliolosa* may be found. At Isachsen the clay plain sloped down to the tide mark without any marked change. The uniformity must be due partly to the salinity of the clay even far from the sea. But the tide is only about one foot, and, although about two square miles of water may open in the head of the bay in late summer, an onshore wind quickly pushes brash ice against the shore. Consequently little or no spray blows ashore.

*Sandy Plains*

Dissected muddy sand plains occur in areas of outcropping sandstone. The high ground in such areas is usually very arid, yielding densely caespitose *Cerastium arcticum*, *Papaver radicatum*, *Saxifraga caespitosa*, and traces of other species. Springy areas on the gully slopes may yield *Phippsia concinna*, *Poa arctica* ssp. *caespitans*, *Luzula confusa*, *L. nivalis*, *Oxyria digyna*, *Cerastium arcticum*, *C. regelii*, *Stellaria ciliatosepala*, *Ranunculus sabinei*, *Papaver radicatum*, *Draba bellii*, and *Saxifraga* spp. This habitat grades into clay plain when both shale and sandstone occur.

*Gypsum Gravel*

Several gypsum domes, the first described by Heywood (1957), occur on Ellef Ringnes. One such outcrop was visited on a small island adjacent to the Christopher Pen. The top and upper slopes were almost completely barren. The shallow gravel slopes at the foot of the hill bore numerous vigorous colonies of *Saxifraga caespitosa* and *S. oppositifolia* and occasional plants of *Arenaria rubella*, but virtually no trace of any other species.

*Marsh*

Fully developed marshes are scarce, because of the lack of continuous moisture supply in many low-lying areas. If all summers were as wet as 1960 marshes would be somewhat commoner, but the frequent occurrence of very dry summers restricts them greatly. Five marshes were seen at Isachsen, ranging in size from a fraction of an acre to nearly five acres. They are dependent upon large snowdrifts for a continuous water supply. Apart from mosses the dominant plants are *Pleuropogon sabinei*, *Dupontia fisheri*, *Eriophorum scheuchzeri*, *Juncus biglumis*, and *Ranunculus hyperboreus*. *Alopecurus alpinus* and *Saxifraga foliolosa* may invade the marshes from drier habitats. *Arctagrostis latifolia* occurred in one marsh only. According to S. D. MacDonald (personal communication) marshy ground is somewhat more abundant northeast of Isachsen on the west side of Louise Fiord. It was thought that a careful search might reveal vegetative plants of, e.g., *Carex misandra*, but no trace could be found of any Cyperaceae other than *Eriophorum scheuchzeri*.

## Parasitic Fungi

A few small fleshy fungi occur in Ellef Ringnes I. and various saprophytic, or at most hemiparasitic, microfungi occur on old leaves and stems. The latter, abundant throughout the arctic, mature gradually during 2 or 3 years and are not conspicuously limited by the severity of the climate. All these

fungi will be considered elsewhere. It is desirable, however, to consider here the strictly parasitic species, which have a less random distribution. Specimens are in DAOM.

MELAMPSORA EPITEA Thuem., Mitth. Forstl. Versuchsw. Oesterr. 2:38 and 40. 1879.

Scarce at Isachsen on *Salix arctica* Pall., *4241A*, *4364*. Recorded north to Alert; one of the commonest arctic rusts.

PUCCINIA HEUCHERAE (Schw.) Diet. var. SAXIFRAGAE (Schlecht.) Savile, Can. J. Botany, 32:408. 1954.

Occasional at Isachsen on *Saxifraga nivalis* L., *4259*, *4300*, *4327*; trace on *S. tenuis* (Wahlenb.) H. Sm., *4285A*. Two specimens previously reported to have been on *S. tenuis* (Savile 1954) proved to have been on stunted *S. nivalis*. The minute sori on the deeply pigmented leaves of the Isachsen plants were visible only under the microscope, and the susceptibility of the host cannot yet be assessed. Larger sori in a recent collection from northwestern B.C. suggest that the somewhat atypical Yukon plant may be more susceptible.

PUCCINIA FISCHERI Cruchet & Mayor, Bull. soc. vaudoise sci. nat. 44:471. 1909.

Small amounts were found in two colonies of *Saxifraga oppositifolia* L. at Isachsen, *4298A*, *4341*, *4365*. In addition to the locations reported by Savile (1959) it was found abundantly in 1959 at Resolute; however, careful searches failed to reveal it at several low-arctic sites. Its occurrence at Isachsen supports the view that it follows its host to the northern limit of land; but it evidently does not reach the southern limit of its host.

USTILAGO VINOSA (Berk.) Tul., Ann. Sci. Nat. Botan. 3, 7:96. 1847.

A single infected clonal colony of *Oxyria digyna* (L.) Hill was found on the Christopher Pen., *4188*. This collection is further north than any previous record. This smut should be found in northern Ellesmere and Peary Land eventually. It is often overlooked because the healthy plants may be nearly as deeply pigmented as the smutted ones.

ARCTICOMYCES WARMINGII (Rostr.) Savile, Can. J. Botany, 37:984. 1959.

Occasional but fruiting sparsely on *Saxifraga oppositifolia* at Isachsen, *4229*, *4298B*, *4304*; and Christopher Pen., *4203A*. This fungus is easily found once the searcher knows what to look for. In addition to the records previously presented, it was found in 1959 at Resolute, Coral Harbour, Frobisher Bay, and (trace only) Great Whale River on *S. oppositifolia*. At Coral Harbour it was also found on *S. aizoides*.

The last three of these fungi are systemic and perennial in their host plants, which enables them to persist even in exceptionally severe seasons. *Melampsora epitea* persists because the urediniospores sift under the bud scales and infect the meristematic tissue, with the result that the leaves emerge bearing nearly mature sori. *Puccinia heucherae* var. *saxifragae* seems to survive by virtue of the semievergreen leaves of *Saxifraga nivalis*, which allow sori that were immature at the onset of winter to resume growth in the spring. Only by some such insurance against disastrously cold or short summers can strictly parasitic fungi persist in regions of such extremely severe climate.

## Vascular Plants

### GENERAL DISCUSSION

As noted under Climate and Weather, the plants from the area under discussion are more severely stunted than those from many localities farther north. The severity of the climate is further reflected in the limited number of species to be found. The following annotated list records 49 species, 48 of which occurred at Isachsen where the high relief gave a good variety of habitats. It is improbable that further searches will add many more species to the northwestern half of Ellef Ringnes I. A few rarities may be added, but rarity in this instance will generally be due to recent arrival and will not seriously affect the phytogeographic picture. Collecting on Amund Ringnes and the southeastern part of Ellef Ringnes should add a few species beyond those here recorded. The few records from the other outer islands, Meighen, Borden, and Mackenzie King, are all of plants that are common on Ellef Ringnes; and it is unlikely that detailed coverage of these islands would materially change the present list. If we exclude Amund Ringnes it is very doubtful if the total flora of the whole group of islands is as high as 60 species. The specialization of the flora to a severe climate is emphasized by the fact that the 11 vascular plants recorded from the high central plateau of Somerset I. (Savile 1959) all occurred at Isachsen and that several (*Alopecurus alpinus*, *Papaver radicatum*, *Draba oblongata*, and *Saxifraga oppositifolia*) were dominant.

Porsild (1957) shows Ellef Ringnes records on his distribution maps for five species not in my list. *Poa glauca* was included on the basis of Simmons' (1913) report of it being taken (without stated location) by Isachsen. Isachsen was evidently not an assiduous botanical collector, for Simmons (1906) credits him with only a few common species from Ellesmere. He credits him with only six species from Ellef Ringnes, the others all being conspicuous and ubiquitous plants: *Cerastium "alpinum"* (*arcticum*), *Papaver radicatum*, *Draba alpina* (including *bellii* in Simmons' usage), *Saxifraga oppositifolia*, and *Potentilla emarginata* (*hyparctica*). It seems strange that the only grass collected by Isachsen should have been one that neither MacDonald nor I could find and for which I made an intensive search because of the published record. If the grass was *Poa glauca* (a misdetermination of the overwintered material is possible) the probability is that it was taken closer to Goose Fiord, perhaps on the east coast of Amund Ringnes or the southern tip of Axel Heiberg. It should be noted that *P. glauca* is unrecorded from a large area in the northwestern part of the Canadian arctic, including such adequately botanized islands as Prince Patrick, Cornwallis, and Somerset. The other four unverified species are *Carex aquatilis*, *Stellaria laeta*, *Saxifraga hirculus*, and *Potentilla vahliana*. The last is based on extremely stunted plants that prove, under the microscope, to be the common *P. hyparctica*. There is no specimen in CAN for any of the other three species, nor can any pertinent notes or literature references be found. A careful search was made for these species and, while *Stellaria laeta* might have been overlooked because of its resemblance to *S. crassipes* and *S. ciliatosepala*, I am confident that the other two plants do not occur near Isachsen. Mr. MacDonald (personal

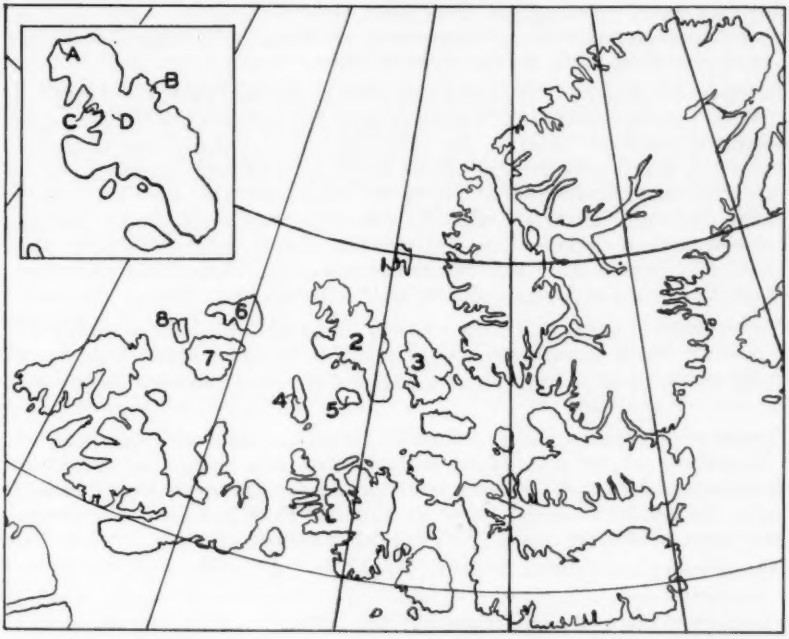


FIG. 1. Queen Elizabeth Is., showing location of: 1. Meighen I.; 2. Ellef Ringnes I.; 3. Amund Ringnes I.; 4. King Christian I.; 5. Lougheed I.; 6. Borden I.; 7. Mackenzie King I.; 8. Brock I. Inset, sites visited on Ellef Ringnes I.: A. Cape Isachsen; B. Christopher Pen.; C. Isachsen; D. sandstone barrens.

communication) is equally certain that *Saxifraga hirculus* is absent from the Louise Fiord marshes visited by him.

The following list incorporates all the records of which specimens have been seen. Numbers unaccompanied by a collector's name represent my specimens, which, together with those of A. E. Collin, D. R. Horn, J. F. McAlpine, and D. St-Onge, are all in DAO. The remaining specimens are in CAN. Isachsen specimens are given first, followed by other Ellef Ringnes records and finally records from other islands, "Sandstone barrens" refers to a weathered sandstone area 10 miles ESE. of Isachsen, visited on July 1 (Fig. 1, inset). The nomenclature of Porsild (1957) is followed to simplify reference to his maps.

## ANNOTATED LIST

ALOPECURUS ALPINUS J. E. Sm., Fl. Brit. 3:1386. 1804.

Common on wet or dry clay or muddy sand and even penetrating marshes. Almost ubiquitous and little stunted except by inadequate water supply. Isachsen, *4239*, *4290*, *4359*, *4360*; Taylor; Heywood *1*; MacDonald *172*, *173*. Christopher Pen., *4187*. Mackenzie King I., Thorsteinsson *58-1*. Overwintered plants abundant on Lougheed I.

PHIPPSIA ALGIDA (Soland.) R. Br., Chlor. Melv. 27. 1823.

Occasional on moist clay or wet sand. Isachsen, *4267B*; MacDonald *184*. Christopher Pen., *4199*. Borden I., D. R. Horn.

PHIPPSIA CONCINNA (T. Fries) Lindeb., Botan. Notiser, 1898:155. 1898.

More abundant than *Ph. algida* at least at Isachsen. Sometimes easily separated from the latter in the field, but stunted plants are often very similar. A mixed collection was made at Isachsen of both extremes and apparent intermediates; however, Dr. W. G. Dore found that they were readily sorted into the two species under the dissecting microscope. On the average, *concinna* seemed to prefer drier habitats than *algida*. Isachsen, *4267A*, *4291*, *4352*; MacDonald *182*, *183*. Sandstone barrens, *4154*. Christopher Pen., *4190*. West coast of Meighen I. (80°00′ N. 100°00′ W.), *4322*.

ARCTAGROSTIS LATIFOLIA (R. Br.) Griseb. in Ledeb., Fl. Ross. 4:434. 1853.

A single colony was found at Isachsen (*4370*) in flooded moss or open water in the deepest part of a marsh. On August 4 the most advanced plants were far short of anthesis.

DESCHAMPSIA PUMILA (Ledeb.) Ostenf., Medd. om Grønl. 44:169. 1923.

Occasional on springy ground or in stream beds, caespitose or matted; largely vegetative and still very immature in early August. Plants brought into a heated building developed slightly but were still far from anthesis on August 8. Isachsen, *4333*, *4377*, *4381*; MacDonald *175*, *176*.

DESCHAMPSIA BREVIFOLIA R. Br. in Parry, 1st Voyage, App. ccxci. 1824.

Immature material evidently of this species was found in muddy sand on a river flood plain on the Christopher Pen., *4195A*.

POA ALPIGENA (Fries) Lindm. var. COLPODEA (Fries) Schol., Skrift. Svalbard og Ishavet, 62:89. 1934.

The Isachsen specimens are a close match for Scholander's illustration, although there is some doubt as to the preferred name for this plant. None of the Isachsen plants are clearly viviparous, probably because of arrested development. Occasional on disturbed, slightly moist, clay slopes. Isachsen, *4351*, *4380*; MacDonald *190*, *191*.

POA ARCTICA R. Br. ssp. CAESPITANS (Simm.) Nannf., Symb. Bot. Ups. 4:35. 1940.

All the Isachsen material has abortive pollen and is laxly caespitose or short-rhizomatous. The plants set no seed in 1953, 1954, 1959, and 1960. In view of the high July temperature in 1959 it appears that nearly all reproduction must be vegetative in this area. Occurs abundantly on springy gravel and clay slopes or moist mossy strips of boulder talus. The stature varies both according to habitat and from clone to clone. Isachsen, *4305*, *4329*, *4346*, *4361*, *4362*, *4375*; MacDonald *189*. Sandstone barrens, *4153*.

POA ABBREVIATA R. Br. in Parry, 1st Voyage, App. cclxxxvii. 1824.

Abundant and well developed on moist muddy slopes or gravel flats. Occasional and more stunted in crevices of clay polygons. Fruited freely, with seed more or less mature by July 31. Isachsen, *4240*, *4287*, *4288*, *4309*, *4332*; MacDonald *186*, *187*, *188*.

PLEUROPOGON SABINEI R. Br. in Parry, 1st Voyage, App. cclxxxix. 1824.

Abundant in the few marshes and occasional in shallow, muddy streams. Plants on moss hummocks or mud have short erect leaves; but the vegetative rosettes of plants in standing water have longer leaves nearly all of which float. The former is evidently what Simmons (1906) refers to as f. *terrestris*, but the difference is plainly ecological only, for there is complete intergradation. Even the most advanced plants came late into anthesis in 1960 and set no seed, partly because the marshes were flooded. MacDonald's collection of August 19, 1954, had not reached anthesis. Isachsen, *4247*, *4316*, *4320*, *4334*; MacDonald *185*.

COLPODIUM VAHLIANUM (Liebm.) Nevski, Fl. U.S.S.R. 2:436. 1934.

Rare and barely into anthesis on August 4 on a springy clay gravel slope. Isachsen, *4372*.

DUPONTIA FISHERI R. Br. in Parry, 1st Voyage, App. ccxci. 1824.

Moderately common in marshes but formed no seed. Isachsen, *4321*, *4337*, *4368*, *4378*; MacDonald *177*.

PUCCINELLIA ANGUSTATA (R. Br.) Rand & Redf., Fl. Mt. Desert I., Maine, p. 181. 1894.

The sole invader of the most alkaline clay plains and common on moist clay or muddy sand. Mostly prostrate but suberect on a solifluction slope where several species were well developed. Set seed in 1959, but not in 1954 or 1960. Isachsen, *4261*, *4350*, *4353*, *4369*; Taylor; Heywood *3*; MacDonald *192*, *193*, *194*. Christopher Pen., *4191*, *4195*.

FESTUCA BRACHYPHYLLA Schultes, Mant. 3:646. 1827.

Common on springy slopes. Rare in sandy areas. Many colonies were checked but no *F. baffinensis* could be found. Isachsen, *4308*, *4354*; Heywood *2*; MacDonald *178*, *179*. Sandstone barrens, *4154A*.

ERIOPHORUM SCHEUCHZERI Hoppe, Botan. Taschenb., 104. 1800.

Abundant locally in two marshes, and fruited freely. Isachsen, *4175*, *4279*, *4318*; Heywood *4*.

JUNCUS BIGLUMIS L., Sp. Pl. ed. 1, 328. 1753.

Scattered in the drier parts of marshes or on wet, muddy slopes. Isachsen, *4176*, *4302*, *4371*; MacDonald *196*, *197*. Christopher Pen., *4198*.

LUZULA CONFUSA Lindeb., Botan. Notiser, 1855:9. 1855.

Common on moist slopes, often associated with *L. nivalis*. Isachsen, *4274*, *4286*, *4306*; MacDonald *199*, *200*. Sandstone barrens *4157*. Christopher Pen., *4200*. Meighen I., Thorsteinsson *57-2*. Mackenzie King I., Thorsteinsson *58-2*.

LUZULA NIVALIS (Laest.) Beurl., Botan. Notiser, 1853:55. 1853.

Common on springy slopes of muddy sand or gravel or on fertile talus slopes. Isachsen, *4276*, *4282*, *4292*, *4307*; MacDonald *203*. Sandstone barrens, *4156*. Mackenzie King I., Thorsteinsson *58-3*.

SALIX ARCTICA Pall., Fl. Ross. 1, 2:86. 1788.

Scarce on springy slopes in moss. Fruited freely in 1959 but sparsely in 1960, and apparently not at all in 1954. All plants seen were very depauperate.

The branches were almost completely buried in moss, with only the leaves and catkins emerging. Ring counts of some of the larger plants at Isachsen showed stems up to about 18 years old in the largest colony and much less in a smaller one. Even the large colony may well be only about 50 years old. The plant from Hoodoo R. in the southern part of the island was nearly 30 years old. Isachsen, *4222*, *4241*; MacDonald *204*. Hoodoo R., 78°15′ N. 100° 05′ W., D. St-Onge.

OXYRIA DIGYNA (L.) Hill, Hort. Kew., 158. 1768.

Widely scattered at Isachsen but usually stunted and sterile except on springy slopes with continuous water supply. Isachsen, *4234*, *4373*; MacDonald *205*. Sandstone barrens, *4145*. Christopher Pen., *4188*. Sandstone area, 78°20′ N. 103°00′ W., D. St-Onge.

POLYGONUM VIVIPARUM L., Sp. Pl. ed. 1, 360. 1753.

A single colony was found south of Isachsen in moss hummocks with *Salix arctica*. The leaves and fruting stems projected only 1.8 to 2.0 cm from the moss, but a few stems had projected nearly 4 cm in 1959. Isachsen, *4301*.

STELLARIA CILIATOSEPALA Trautv. in Middend., Reise in d. äussersten Norden u. Osten Sib. 2:52. 1856.

Moderately common but flowering sparsely on moist gravel, mud, or talus slopes. Best developed in fertile strips of talus with *Poa arctica* ssp. *caespitans*. No seed produced in 1954 or 1960. Isachsen, *4254*, *4268A*, *4328*, *4376*; MacDonald *207*, *208*. Sandstone barrens, *4151*. Christopher Pen., *4186*.

STELLARIA CRASSIPES Hult., Botan. Notiser, 1943:261. 1943.

Occasional in the same habitats as *S. ciliatosepala*, and sometimes mixed with it. Isachsen, *4253*, *4268*, *4376A*. ?Borden I. (vegetative), D. R. Horn.

CERASTIUM ARCTICUM Lange, Fl. Dan. 50:7. 1880.

Common on mud, sand, or gravel slopes, especially where moisture regularly available. Isachsen, *4138*, *4215*, *4340*; Taylor; Heywood *5*; MacDonald *209*, *210*, *211*. Sandstone barrens, *4149*. Christopher Pen., *4189*. Borden I., D. R. Horn. Mackenzie King I., Thorsteinsson *58-4*.

CERASTIUM REGELII Ostenf., Christiania Videnskab. Selskab. Skrifter, I, Mat.-Nat. Kl. No. 8 (1909):10. 1910.

Moderately common, forming dense vegetative mats on moist sand or clay slopes. Isachsen, *4260*; Heywood *6*; MacDonald *212*, *213*. Sandstone barrens, *4150*. Christopher Pen., *4192*.

ARENARIA ROSSII R. Br. in Franklin, Narr. 1st Journ., App., 738. 1823.

Scarce on springy slopes. Isachsen, *4242*, *4303*, *4358*.

ARENARIA RUBELLA (Wahlenb.) J. E. Sm., Engl. Botan., Suppl. 1, tab. 2638. 1831.

Widespread but never abundant on springy slopes. Isachsen, *4269*, *4296*; MacDonald *206*. Christopher Pen., *4204*.

RANUNCULUS HYPERBOREUS Rottb., Skrifter Kjøbenhavnske Selskab. Laerd. Videnskab. 10:458. 1770.

Abundant in one marsh, but flowered sparingly perhaps because of the

continuously high water level. Apparently did not mature any fruit. Isachsen, *4248, 4317*; MacDonald *214*.

RANUNCULUS SABINEI R. Br. in Parry, 1st Voyage, Supp. cclxiv. 1824.

Common on slightly moist clay plains and slopes. Occasional on sandy soil. Fruited freely, with many achenes mature by July 26. Isachsen, *4131, 4140, 4218, 4238, 4313*; Taylor; Heywood *8*; MacDonald *215, 216, 217, 218*. Sandstone barrens, *4144*. Christopher Pen., *4185*.

RANUNCULUS NIVALIS L., Sp. Pl. ed. 1, 553. 1753.

Occasional on springy slopes. Isachsen, *4130, 4220, 4367*.

RANUNCULUS SULPHUREUS Sol. in Phipps, Voy. N. Pole, 202. 1774.

In habitat of *R. nivalis* but scarcer, in part because seed matured more slowly. Isachsen, *4221, 4228, 4366*; MacDonald *219, 220*. Mackenzie King I., Thorsteinsson *58-6*.

PAPAVER RADICATUM Rottb., Skrifter Kjøbenhavnske Laerd. Videnskab. 10:445. 1770.

Very common on clay, moderately so on muddy sand or Beaufort gravel. Fruiting stems mostly 6–10 cm high; but to 14.5 cm on one fertile and protected slope, where the 1959 stems reached 20 cm. On exposed sites the flower buds remained sessile until the flowers opened. In addition to the typical form with lemon yellow petals, a form with petals almost white except for a yellow claw occurred in most colonies; it usually formed less than 10% of the population but rarely approached 20%. See Porsild (1955) for a discussion of variation in this species. Isachsen, *4245, 4382* (pale form), *4162, 4244, 4281, 4383* (normal form); MacDonald *222, 223* (pale), *221, 224* (normal); Heywood *10*. Sandstone barrens, *4155*. Christopher Pen., *4184*. Meighen I., Thorsteinsson *57-4, 57-8*. Borden I., D. R. Horn. Mackenzie King I., Thorsteinsson *58-7*.

COCHLEARIA OFFICINALIS L., Sp. Pl. ed. 1, 647. 1753.

Occasional on wet mud or muddy sand and gravel, often several miles inland. The sites were always disturbed by solifluction or outwash. Isachsen, *4236, 4270, 4272, 4297*. Christopher Pen., *4196*.

CARDAMINE BELLIDIFOLIA L., Sp. Pl. ed. 1, 654. 1753.

Occasional on wet mud or gravel slopes. Fruited freely in 1959, but few siliques were approaching maturity in early August 1960 and seed production was probably small. Isachsen, *4168, 4357*; MacDonald *231, 232, 233, 234*. Sandstone barrens, *4146*. Christopher Pen., *4205*.

DRABA ALPINA L., Sp. Pl. ed. 1, 642. 1753.

Occasional on clay plains or slopes, usually in mossy cracks. Isachsen, *4214, 4232, 4265, 4310, 4312*.

DRABA BELLII Holm in Fedde, Rept. Spec. Nov. 3:338. 1907.

Common on sandy or gravelly sites, where it forms large rosettes with a long tap root. Apparently absent from clay. Isachsen, *4235*. Sandstone barrens, *4152A*. Christopher Pen., *4180, 4181, 4182, 4206*. Fifteen miles NW. of Isachsen, D. St-Onge. ?Mackenzie King I., Thorsteinsson *58-9* (very young).

DRABA LACTEA Adams, Mem. soc. nat. Mosc. 5:104. 1817.

Rare in moist moss–lichen patches, very stunted. Isachsen, *4243*, *4295*.

DRABA OBLONGATA R. Br. in Ross, Voy. Disc., App., cxliii. 1819.

Abundant on moist to moderately dry clay. Very variable in appearance and stature. Petals at first deep yellow and small, but becoming pale and larger in age. Isachsen, *4129*, *4165*, *4166*, *4167*, *4213*, *4217*, *4231*, *4311*; Taylor; Heywood *11*; MacDonald *225*, *226*, *227*, *228*. Sandstone barrens, *4152*. Meighen I., Thorsteinsson *57-5*.

DRABA SUBCAPITATA Simmons, Vasc. Pl. in Fl. Ellesmereland, 87. 1906.

Moderately common on clay or sandy soil. Isachsen, *4164*, *4230*, *4264*; Heywood *65*; MacDonald *229*, *230*. Sandstone barrens, *4152B*. Christopher Pen., *4183*. Mackenzie King I., Thorsteinsson *58-8*.

The determination of the stunted *Draba* specimens from this region, often fruiting at less than 3 cm height, is very difficult. We have in some instances grouped together collections that seem to show some genetic distinction. The changes in petal color and weathering away of some of the pubescence as the season advances are well-known complications; but, when all plants are so stunted that specific distinctions of habit largely disappear, the problem of identification is greatly magnified.

SAXIFRAGA CAESPITOSA L., Sp. Pl. ed. 1, 404. 1753.

Common on rocky and gravelly slopes, but very stunted except on sheltered and fertile sites. Isachsen, *4137*, *4246*, *4283*; Heywood *12*; MacDonald *235*. Christopher Pen., *4202*. Mackenzie King I., Thorsteinsson *58-9*.

SAXIFRAGA CERNUA L., Sp. Pl. ed. 1, 403. 1753.

Common on sandy soil, Beaufort gravel, clay plains, and rocky slopes, if not too arid. Very small and not flowering where exposed to wind or with inadequate water. Generally 4–7 cm high on moist slopes or to 10 cm if sheltered by boulders. Isachsen, *4263*, *4294*, *4331*; Taylor; Heywood *13*, *14*; MacDonald *236*, *237*, *238*. Christopher Pen., *4193*. C. Isachsen, *4326*. Borden I., D. R. Horn. Mackenzie King I., Thorsteinsson *58-13*.

SAXIFRAGA FLAGELLARIS Willd. ssp. PLATYSEPALA (Trautv.) Porsild, Botan. Tidsskr. 50:16. 1954.

Common on moist gravelly mud and occasional on clay plains. Not noted in sandy areas. Flowers freely but seems to produce little seed. Isachsen, *4225*, *4237*, *4299*, *4356*; Heywood *15*; MacDonald *239*.

SAXIFRAGA FOLIOLOSA R. Br. in Parry, 1st Voy., App. 275. 1824.

Scattered on springy slopes and flood plains or in marshes. No flowers formed in 1954, 1959, or 1960. Isachsen, *4257*, *4319*; MacDonald *240*, *241*. Christopher Pen., *4197*, *4207*.

SAXIFRAGA NIVALIS L., Sp. Pl. ed. 1, 401. 1753.

Common on wet to moderately dry gravel and mud slopes. Isachsen, *4163*, *4223*, *4300*, *4338*; Taylor; Heywood *18*; MacDonald *242*, *244*, *245*, *246*, *247*. Sandstone barrens, *4148*. Christopher Pen., *4194*. Mackenzie King I., Thorsteinsson *58-11*.

SAXIFRAGA OPPOSITIFOLIA L., Sp. Pl. ed. 1, 402. 1753.

Common on slightly wet rocky or gravelly slopes, but scarce on clay. In addition to the typical form with strongly campanulate, magenta petals, a form with spreading, long-clawed, pale lilac petals occurred sporadically. The latter variant is perhaps partly adapted to dipterous pollinators and is probably inadaptive where bumblebees are plentiful. Unfortunately, the distinctions in color and morphology are usually difficult to see in pressed specimens, and the distribution of this form is uncertain. However, I have not seen it in the southern arctic and suspect that it is predominantly far northern. The confusion in the nomenclature within *S. oppositifolia*, which has resulted from the failure to distinguish between genetic and ecological variation, is such that I have made no attempt to identify this variant. Isachsen, *4142* (mixed), *4344* (lilac), *4128*, *4298*, *4343* (magenta); MacDonald *248*. Sandstone barrens, *4143* (magenta). Christopher Pen., *4203A* (lilac), *4203B* (magenta). Northeast Amund Ringnes I., A. E. Collin (magenta). Meighen I., Thorsteinsson *57-6*, *57-10*. Borden I., D. R. Horn. Mackenzie King I., Thorsteinsson *58-10*.

SAXIFRAGA RIVULARIS L., Sp. Pl. ed. 1, 404. 1753.

Scattered on moist mud or gravel slopes. Isachsen, *4212*, *4256*, *4284*; Heywood *17*; MacDonald *249*, *250*. Sandstone barrens, *4147*.

SAXIFRAGA TENUIS (Wahlenb.) H. Sm. in Lindm. Svensk Fanerogamfl., 300. 1918.

Common at Isachsen on moderately to very wet mud or gravel slopes; generally with *S. nivalis* but not invading the driest sites of that species. In the high arctic this species is easily told in the field from stunted *nivalis*, but the distinctions are not so clear with the more southerly populations. *S. tenuis* is usually smaller and with a more slender scape, and usually has a few-flowered obconic panicle rather than the nearly spherical panicle of *nivalis*; but these are quantitative characters that do not always hold in stunted plants. The petals of *tenuis* are deep pink outside or generally on both surfaces, those of *nivalis* nearly white (may be tinged pink on back). Calyces and scapes of *tenuis* are always deep red, those of *nivalis* green to red. The scape hairs of *tenuis* are short, straight, conspicuously glandular, and bright pink when fresh (dark purple when dry), those of *nivalis* are longer, laxer, less conspicuously glandular, and white when fresh except for the reddish or brownish glands; this difference in the color of the scape hairs is conspicuous in the field. The leaves of *tenuis* are always sessile and strictly cuneate-spatulate; those of *nivalis* contract abruptly to a short, winged petiole. In small plants it may be necessary to pull a leaf away from the rosette to see the base, but this is the most reliable character, especially in plants dried without heat in which oxidation may obscure hair color. Finally, there are at most short, rusty hairs, often marginal, on leaves of *tenuis*, but generally long, coarse, and conspicuous rusty hairs and a few white ones on those of *nivalis*.

I have been through all the pertinent material in DAO and CAN, and it now appears that we have three disjunct populations of *S. tenuis* in North America, at least two of which are appreciably distinct (Fig. 2I). The high-

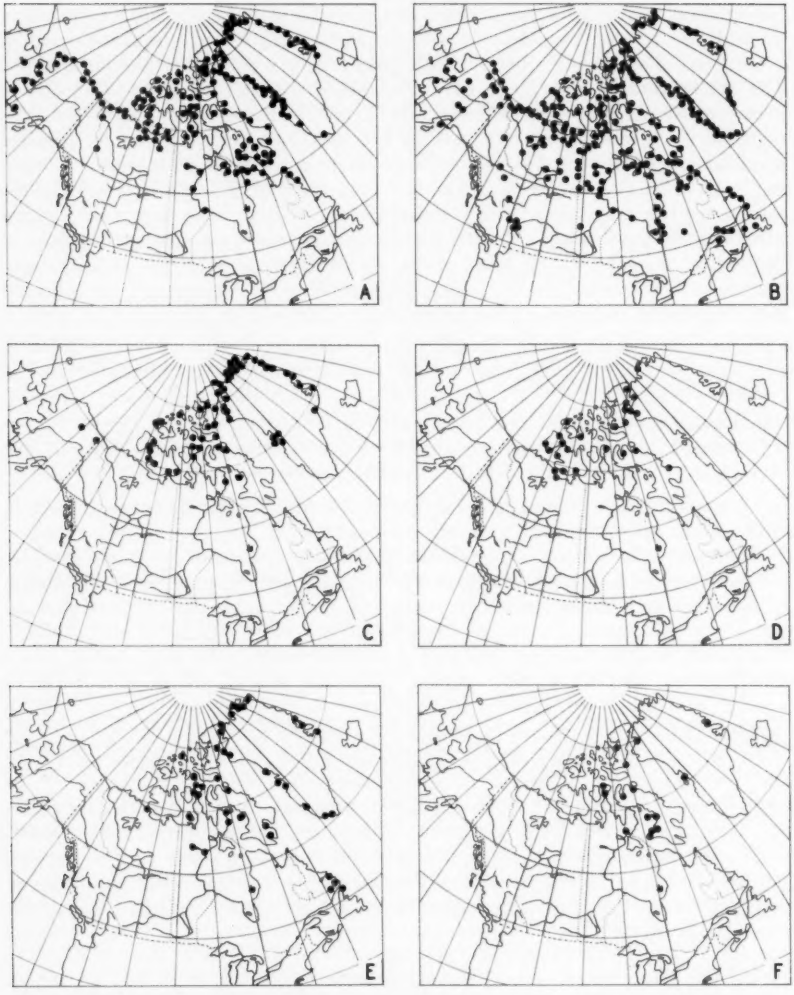


FIG. 2. Nearctic plant distribution patterns. A. *Alopecurus alpinus* (full arctic distribution). B. *Dryas integrifolia* (widespread with gap in NW. islands). C. *Poa abbreviata* (full high arctic). D. *Taraxacum hyparcticum* (high arctic with gap). E. *Stellaria crassipes* (widespread eastern arctic, reaching Ellef Ringnes). F. *Poa arctica* var. *vivipara* (widespread eastern arctic, not on Ellef Ringnes).

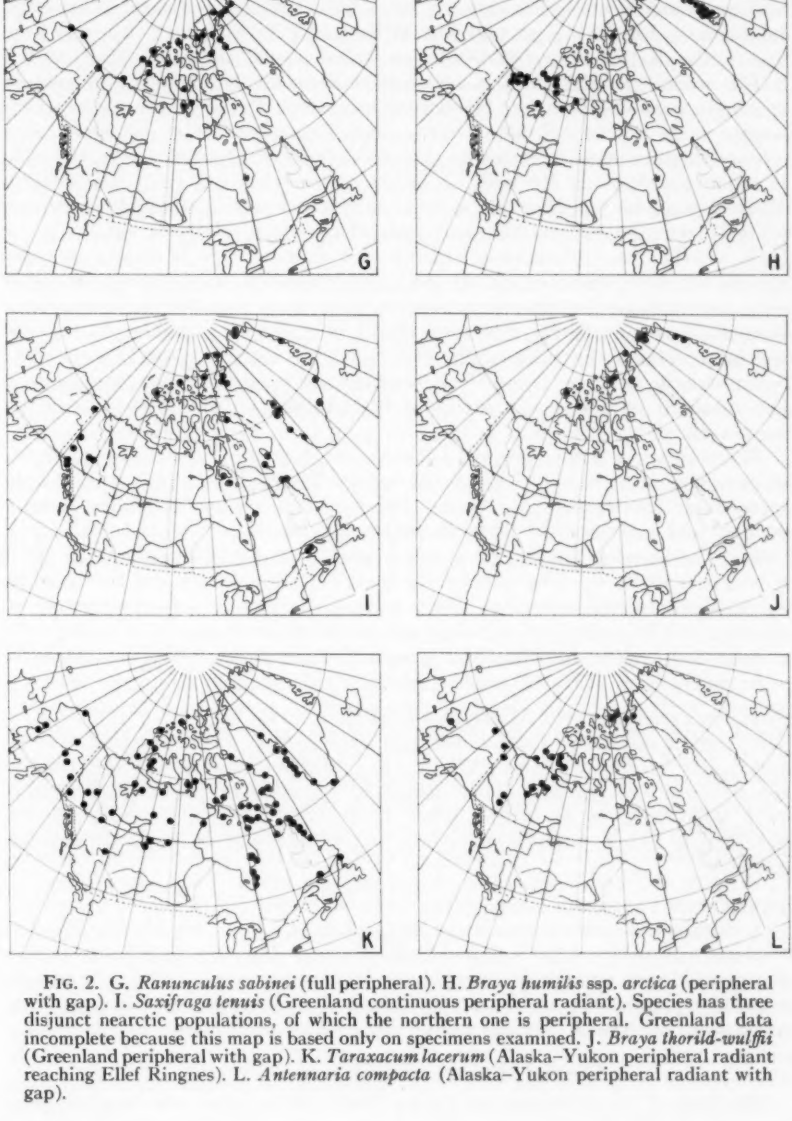


FIG. 2. G. *Ranunculus sabinei* (full peripheral). H. *Braya humilis* ssp. *arctica* (peripheral with gap). I. *Saxifraga tenuis* (Greenland continuous peripheral radiant). Species has three disjunct nearctic populations, of which the northern one is peripheral. Greenland data incomplete because this map is based only on specimens examined. J. *Braya thorild-wulffii* (Greenland peripheral with gap). K. *Taraxacum lacerum* (Alaska–Yukon peripheral radiant reaching Ellef Ringnes). L. *Antennaria compacta* (Alaska–Yukon peripheral radiant with gap).

arctic population is identical with specimens from northern Greenland and seems to have spread from there since the Pleistocene. It occurs near Alert and on Ward Hunt I. in northern Ellesmere, Eureka in western Ellesmere, Isachsen on Ellef Ringnes, and Mould Bay on Prince Patrick. Reports from Cornwallis, Bathurst, and Banks Is. are based on stunted *S. nivalis*.

The second population of *S. tenuis* is centered on Hudson St., but occurs sparingly northward to Eric Harbour in northeast Baffin and has a southern outlier in the Shickshock Mts. (*S. gaspensis* Fern.), the latter evidently being a post-Pleistocene relict. Other specimen localities are Coral Harbour, Coats I., Richmond Gulf, Port Burwell, C. Chidley, Frobisher Bay, and Blacklead I. Most of these plants show less anthocyanin than those of the high arctic and some are virtually without it; and most of them are relatively tall. It is not clear whether the difference in stature and pigmentation is wholly environmental or partly genetic; but, if the gap between the two populations is as large as the available specimens suggest, they have probably been separated since the last interglacial, and some distinction may be expected. In some of the larger plants the sides of the leaf bases are slightly concave instead of being strictly cuneate. One pale Greenland specimen seen may belong here, but material on hand is inadequate for analysis of the whole Greenland population.

The third population is that of the Alaska–Yukon refugium, which seems to have spread little since the last glaciation. These plants are usually deeply pigmented but occasionally green. However, the scape hairs are usually whitish and intermediate in form between those of *nivalis* and high-arctic *tenuis*. The leaves are spatulate but may have short rusty hairs beneath. This population seems to differ genetically from both the others and may also be variable within itself. Because of the variability seen in palearctic *tenuis* no attempt will be made here to name any of the populations.

Recorded on the northwestern Queen Elizabeth Is. only from Isachsen, *4219*, *4224*, *4275*, *4285*, *4293*, *4355*; MacDonald *251*, *252*.

POTENTILLA HYPARCTICA Malte, Rhod. 36:177. 1934.

Abundant on mossy talus slopes and springy gravel. Occasional, stunted, and largely sterile on mossy cracks in clay. Rare on sandy soil and usually sterile. Leaf morphology often abnormal on unfavorable sites. Isachsen, *4139*, *4170*, *4172*, *4226*, *4252A*, *4262*, *4289*, *4339*, *4363*, *4379*; Heywood *19*; MacDonald *253*, *255*. Christopher Pen., *4201*. Mackenzie King I., Thorsteinsson *58-4*.

TARAXACUM LACERUM Greene, Pittonia 4:230. 1901.

A single small colony was found at Isachsen by J. F. McAlpine, July 15, 1960, on a gravelly south slope overlooking a bay. Repeated searches in similar habitats failed to reveal any other plants. This predominantly low-arctic species is evidently spreading northeastward along the rim of the archipelago. It is widespread on Banks I. and is known from Prince Partick I.

## Glacial History and Phytogeography

The flora of the northwestern Queen Elizabeth Is. must now be considered in relation to the glacial history of the region and the whole problem of nearctic

phytogeography. Now that the most fertile part of the region has been studied in some detail, more reliance may be put upon the recorded distribution patterns than was previously possible. Several features of the flora command attention: (1) There are no plants endemic to the region; all the presumptive nearctic endemics are absent or also occur on the mainland to the southwest, in northern Greenland to the northeast, or both. (2) The plants are almost all very severely stunted. (3) The number of species present is very small. (4) Several species are so localized and occur in such small colonies as to suggest that they have reached the region very recently. (5) Numerous species that occur throughout the rest of the archipelago are absent from these islands. (6) Some species occur, in the archipelago, solely or mainly along the northwestern rim; a high proportion of these peripheral species are also lacking from our area.

Let us consider these points in relation to the contemporary climate and the apparent glacial history of the region. Heywood (1957) discusses the glacial history of the Isachsen area, and Craig and Fyles (1960) give an up-to-date discussion of the glaciation of the entire archipelago. Direct examination and aerial photography together have at last given us a picture of glacial history in the archipelago that is relatively complete. As mentioned at the beginning of this paper, there is no convincing evidence of Wisconsin glaciation on any of the islands with which we are concerned or on parts of several islands to the southwest. There has been substantial marine submergence, up to about 180 ft on Ellef Ringnes, but this need not have been due to subsidence under a heavy ice sheet. There is reason to believe that it dates from the high sea level of the last interglacial, when most or all of the continental ice had melted.

Although Ellef Ringnes is devoid of convincing signs of Wisconsin glaciation, this does not necessarily mean that it was ice-free. The reliable physical signs of glaciation (planing and plucking, grooving, striations, moraines, erratics, etc.) are the result of an actively moving ice sheet. For ice to flow it must be under substantial pressure, which is produced only by a very thick accumulation. If, by glaciation, we mean an active continental ice sheet several thousands of feet in thickness, then Ellef Ringnes was almost certainly not glaciated. Quite rightly the glaciologist limits the term to this usage. However, the biologist is interested in the possible persistence of plants and animals in an area. If the area is completely covered by snow and ice for a number of years all life is eradicated from it; and the biologist is understandably inclined to term it glaciated. From his standpoint the main difference between ice sheets 100 ft and 10,000 ft thick is that the thin sheet disappears very quickly when a warming trend is initiated, allowing the land to be recolonized long before the heavy ice sheet has vanished from an adjacent region. There is thus a semantic problem involved, for we lack a definitive term to apply to land that becomes fully covered by snowfields or thin, essentially stationary ice sheets. In the present discussion I shall use the term snow-covered in this sense, although it will be understood that the snow inevitably becomes compacted into a layer of ice as the years pass and new snow continues to accumulate.

For plants to persist, some parts of the land must be freed of snow and

ice for at least a few weeks each year. The size of the flora will depend chiefly upon the length of the snow-free period, the temperature at the ground surface during this period, and, of course, the plants that are available for colonization of the area. The extent of the snow-free area is also important, for small nunataks emerging from a snowfield have a low summer temperature, except on windless days, and the plants on them are apt to be destroyed by snow abrasion in winter. Such small nunataks are likely at best to harbor a few lichens. Thus, if the northwestern Queen Elizabeth Is. were covered during the Wisconsin by persistent snowfields, ranging from a few feet to a few hundred feet in thickness, from which a few hilltops intermittently emerged, they could not have served as a refugium for vascular plants.

We have also to consider the effect of a slight drop in summer temperature and a shortened growing season, even in the absence of permanent snow. When the July mean temperature is about 38° F a change of only a degree in either direction greatly affects the flora. The plants of Isachsen are fewer and more stunted than those of Alert and Mould Bay, where the temperature difference is scarcely measurable. On the central plateau of Somerset I., where the July mean temperature is probably ca. 37° F, only 11 vascular plants could be found. A reduction of the mean summer air temperature by 2° F, accompanied by increased cloud cover, which would reduce the soil surface temperature, would certainly eliminate three-quarters of the higher plants that now grow at Isachsen. It might, indeed, eliminate all of them, leaving only a limited number of mosses and lichens.

After spending a nearly typical summer at Isachsen I find it difficult to believe that any climatic deterioration (due to increased snowfall, lower summer temperature, decreased insolation, or a combination of these factors), sufficient to initiate a continental ice sheet on Ellesmere and Axel Heiberg, could fail to leave this region snow-covered. Even today, with its abundant ground ice and with numerous permanent snowdrifts extending down virtually to sea level, the Isachsen area seems to be on the verge of permanent snow cover; and 100 miles to the northeast we have an icecap on low-lying Meighen I. A modest increase in snowfall, coupled with increased summer cloudiness, would quickly build up the residual drifts until they began to coalesce. Then decreased light absorption would reduce surface heating, both through less ground being bare and through the snow surface being cleaner. The change would thus tend to accelerate, and an essentially complete cover might well develop within 100 years. Because these islands lack high mountains, the snowfall would inevitably be much lighter than on Ellesmere and Axel Heiberg; and only shallow snow- and ice-fields would develop on them, perhaps averaging 500 ft instead of 5000 ft in thickness.

In this connection two features of the Isachsen area should be noted. First, gully formation and the elaboration of drainage patterns in the clay soil has not proceeded very far. Even allowing for low precipitation, I find it difficult to believe that a pattern dating from the end of the last interglacial, when the sea level dropped, could be so extremely immature. To emphasize the fact that erosion is currently proceeding quite fast, I may say that during the spring runoff the flow down the gulleys is strong enough to carry chips of shale weighing more than a gram out onto vegetation or snow patches at

a stream mouth. The second point concerns frost-wedging of bedrock. Although frost action is severe on the cliffs, which consequently are largely talus, the situation is very different on some of the hilltops. When walking over such hilltops I not infrequently found essentially undisturbed bedrock. Fraser (1959) points out that such mechanical weathering is largely correlated with the frequency of freeze–thaw days, and that such frequencies may be three to five times as high in southern Canada as in the Arctic Archipelago. Nevertheless we repeatedly see that frost-wedging does proceed actively in the arctic. If these hilltops had really been continually exposed to frost action since the last interglacial it is difficult to believe that they would show, if anything, less frost action than somewhat similar (but definitely glaciated) exposures in, e.g., northern Newfoundland or the Bruce Peninsula. The relatively undisturbed condition of these surfaces suggests, instead, that they were protected from violent fluctuations by a blanket of snow throughout the Wisconsin and perhaps intermittently since then.

With the onset of the postglacial xerothermic, when the temperature was substantially (perhaps 4° F) higher than today, the whole of this thin cover of snow and ice must have melted quickly, *including the cap on Meighen I.* Even today, with the climate substantially more severe than in the xerothermic, the Meighen icecap is shrinking so rapidly that according to Mr. K. C. Arnold (personal communication) it will vanish within 200 years if the present trend continues. Probably this cap covered the whole of Meighen I. a few hundred years ago. When Mr. Arnold concludes his studies of this icecap it is expected that we shall have a clearer picture of its history. During the last important climatic deterioration there must have been a substantial accumulation of snow and ice on the other islands of this group; and, on such land as was not fully snow-covered, summer conditions, aggravated by the nearness of snow-covered land, must have been too severe to allow survival of most of the vascular plants that occupy the islands today. (A temperature drop of 2° F would probably make conditions slightly worse than those on the Somerset I. plateau today.)

These deductions infer that many of the plants which colonized the northwestern islands during the xerothermic have since been eliminated from them. To analyze the distribution patterns of the arctic plants we must group them into classes, bearing in mind that the groupings are only approximate since no two species show precisely identical distribution patterns. Since we are concerned only with the nearctic, i.e. Greenland, the Canadian arctic, and parts of Alaska, the plants may be considered in the following categories.

*(A) Full arctic.*—Plants found virtually throughout the archipelago and Greenland on ice-free land. This pattern may be typified by *Alopecurus alpinus* (Fig. 2A). Also falling into this class are: *Phippsia algida, Arctagrostis latifolia, Pleuropogon sabinei, Colpodium vahlianum, Dupontia fisheri, Festuca brachyphylla, Eriophorum scheuchzeri, Juncus biglumis, Luzula nivalis, L. confusa, Salix arctica, Oxyria digyna, Polygonum viviparum, Stellaria ciliatosepala, Arenaria rossii, A. rubella, Ranunculus hyperboreus, R. nivalis, Papaver radicatum, Cochlearia officinalis, Cardamine bellidifolia, Draba alpina, D. bellii, D. lactea, Saxifraga caespitosa, S. cernua, S. foliolosa, S. nivalis, S. oppositifolia, S. rivularis,* and *Potentilla hyparctica* (32 taxa).

*(B) Widespread with NW. gap.*—These plants have a pattern similar to (*A*) except that they are absent from the northwestern islands. The gap may include only the islands dealt with in this paper or it may extend southeastward to Cornwallis or even Somerset I. It is typified by *Dryas integrifolia* (Fig. 2B). Other examples are: *Woodsia glabella, Cystopteris fragilis, Equisetum arvense, E. variegatum, Lycopodium selago, Trisetum spicatum, Poa glauca, Puccinellia phryganodes, Festuca baffinensis, Eriophorum triste, Kobresia myosuroides, Carex nardina, C. rupestris, C. aquatilis* var. *stans, C. misandra, C. membranacea, Stellaria monantha, S. laeta, Silene acaulis* var. *exscapa, Melandrium apetalum* ssp. *arcticum, M. affine, Ranunculus pedatifidus, Eutrema edwardsii, Cardamine pratensis* var. *angustifolia, Lesquerella arctica* var. *arctica, Draba nivalis, D. groenlandica, Braya purpurascens, Saxifraga hirculus, S. tricuspidata, Potentilla pulchella* var. *gracilicaulis, P. vahliana, Epilobium latifolium, Cassiope tetragona, Pedicularis capitata, P. arctica, P. lanata, Erigeron eriocephalus, Arnica alpina* ssp. *angustifolia*, and *Taraxacum phymatocarpum* (41 taxa). A few of these species do not show complete Greenland distribution, but they occur in northernmost Greenland. On the other hand, I have excluded several species that are recorded substantially north of Ellef Ringnes but not in extreme northern Ellesmere or Greenland.

*(C) Full high-arctic species.*—These differ from (*A*) in being scarce or lacking in the southern arctic. Typified by *Poa abbreviata* (Fig. 2C). Other species are: *Phippsia concinna, Deschampsia brevifolia, Puccinellia angustata, Cerastium arcticum, C. regelii, Ranunculus sulphureus, Draba subcapitata*, and *D. oblongata* (nine taxa). *Phippsia concinna* is included with some reservation. It is included by some authors with *P. algida*, and its full distribution is uncertain.

*(D) High arctic with gap.*—As (*C*) except lacking in the northwestern islands. Typified by *Taraxacum hyparcticum* (Fig. 2D). Other species are *Poa hartzii, Puccinellia andersonii*, and *Lychnis triflora* (four taxa). This is a small group, but it is significant that any of these high-arctic species should show the gap.

*(E) Widespread eastern arctic, reaching Ellef Ringnes.*—These plants occur from the mainland to northern Ellesmere but have scarcely invaded the western arctic. Typified by *Stellaria crassipes* (Fig. 2E). Other examples are *Deschampsia pumila* and *Poa arctica* ssp. *caespitans* (three taxa).

*(F) Widespread eastern arctic, not on Ellef Ringnes.*—Typified by *Poa arctica* var. *vivipara* (Fig. 2F). Other species are *Cerastium alpinum* and *Pedicularis hirsuta* (three taxa).

*(G) Full peripheral species.*—These plants occur in Greenland and in Yukon, but in the archipelago they are largely confined to the northwestern periphery, extending along it without a conspicuous break. Their pattern does not tell us whether they originated from Greenland or the mainland. Typified by *Ranunculus sabinei* (Fig. 2G). Other examples are *Poa alpigena* var. *colpodea* and *Saxifraga flagellaris* ssp. *platysepala* (three taxa).

*(H) Peripheral with gap.*—As (*G*) except absent from the northwestern islands. Typified by *Braya humilis* ssp. *arctica* (Fig. 2H). Other examples are: *Erysimum pallasii, Erigeron compositus*, and *Taraxacum pumilum* (four taxa). *Polemonium boreale*, spreading from Alaska to Melville I., and with a

localized population in east Greenland, is excluded from this group, because the latter population may be of palearctic origin.

*(I) Greenland continuous peripheral radiants.*—Plants originating from northern Greenland and spreading without important gaps along the outer islands. Represented only by the high-arctic population of *Saxifraga tenuis* (Fig. 2I). See discussion in annotated list. The Greenland localities are incomplete, being based only on specimens seen personally.

*(J) Greenland peripheral radiants, with gap.*—As (*I*) except absent from the northwestern islands. Represented only by *Braya thorild-wulffii* (Fig. 2J).

*(K) Alaska–Yukon peripheral radiants reaching Ellef Ringnes.*—Represented only by *Taraxacum lacerum* (Fig. 2K). This species is widespread in the low arctic and subarctic. It occurs widely in the interior of Alaska and may have spread through the rest of its current range from there in postglacial times. It has, of course, great potentiality for spread by wind; and most of its range is in the belt of strong prevailing WNW. winds. In the archipelago it is peripheral, and it apparently reached Ellef Ringnes very recently.

*(L) Alaska–Yukon peripheral radiants, with gap.*—These are species that evidently spread from the Alaska–Yukon refugia; they are absent from northernmost Greenland, although two have just reached northwestern Greenland. Typified by *Antennaria compacta* (Fig. 2L). Other species are: *Geum rossii* and *Androsace septentrionalis* (three taxa).

*(M) Alaska–Yukon radiants of limited spread.*—Some are primarily peripheral, others have spread strongly eastward. Five species have reached Prince Patrick I.: *Caltha palustris* var. *arctica*, *Ranunculus gmelini*, *Parrya arctica*, *Oxytropis arctica*, and *Petasites frigidus*. More than twenty other species show smaller penetration of the archipelago. These plants have no bearing on the present study and are therefore not listed.

The maps illustrating these classes are based on those of Porsild (1957), modified where necessary. The distributions of the other species listed may also be seen by reference to Porsild's maps.

Summarizing the above categories, we find that in the six classes of the Ellef Ringnes flora (*A*, *C*, *E*, *G*, *I*, *K*) we have 49 species. In the six corresponding classes of plants that might be expected there (*B*, *D*, *F*, *H*, *J*, *L*) we have 56 species; or, if we add the 5 plants of category M that are on Prince Patrick I., a total of 61. Thus the region contains less than half the plants to be expected if spread were unrestricted. There being no important physiographic barriers to restrict immigrants, this is an astonishingly uneven distribution. The explanation unquestionably lies, in part, in the present severity of the climate; but there is good reason to believe that a number of species have been eliminated from the northwestern islands by postglacial climatic deterioration. Many of the plants in class *B* must have advanced into the archipelago over a broad front after the ice receded, and some of them may have simply not yet reached the northwestern islands. Nevertheless it is a suspicious circumstance that these plants have spread all round this region. It is also significant that 4 out of the 13 high-arctic species (classes *C* and *D*) surround the northwestern islands but are absent from them. There is no question here of lack of time. Most important of all in assessing the history of the northwestern islands are the peripheral plants. Here we have five

species (*G*, *I*, *K*) in the northwestern islands and eight species (*H*, *J*, *L*) northeast and southwest of these islands but not in them. It is possible that some of the five species in class *M* really should be added to this group, but we cannot decide the point. The only rational explanation for this broken peripheral pattern seems to be that it was complete in the xerothermic and has been broken since. We shall return to this point in discussing the presumptive endemics of the archipelago.

The data presented by Ahlmann (1953) for glaciers in Norway, Sweden, and Iceland suggest that the xerothermic lasted from about 5000 to 1000 B.C.; and that subsequent climatic deterioration reached peaks at about 500 B.C. and 1700 A.D., the latter being the more severe. The period between these two peaks seems to have been substantially cooler than the xerothermic and probably no warmer than today. There is presumably a substantial lag between climatic change and change in the limits of a large glacier, which would not be applicable to thin snowfields such as are believed to have covered the peripheral islands. It is accordingly suggested that the xerothermic set in between 6000 and 5000 B.C., that the first postglacial cold period lasted from ca. 1000 B.C. to 0 A.D., and that the second cold period began about 1000 A.D. with little improvement occurring before 1600 (reflected in glacier recessions after 1700). Although there is no assurance that the climatic fluctuations were everywhere contemporaneous, it is encouraging to note that Brodkorb (1960), who recently summarized the records of bones of northern birds in Florida middens, indicates cold periods in North America at ca. 1000 B.C. and 1000–1700 A.D. Zeuner (1950) suggests that the xerothermic was not simultaneous everywhere, but his data support a deterioration not later than 1000 B.C. Ahlmann also discusses the recent recession of various European and North American glaciers. Despite individual variations, almost all show a rapid recession during the last 50 to 100 years and the more northerly ones show a relatively consistent decline for more than 200 years. Thus we have a reasonably coherent picture of northern postglacial climatic fluctuations upon which to try to reconstruct the botanical history of the northwestern islands.

At the start of the xerothermic the thin ice cover on the northwestern periphery of the archipelago must have melted rapidly. At this time a large proportion of the ice would still have been present in the continental ice sheets, the sea level would have been substantially below where it stands today, and there would have been fewer and narrower channels between the outer islands. Some species of plants seem to have spread quite rapidly along this peripheral strip. A similar rapid coastwise migration in British Columbia at the end of the Pleistocene has been indicated by Calder and Savile (1959, 1960). Coastwise spread of plants often tends to be rapid because winds tend to blow along a coast. Spread by animals may also have been a factor. At this time there seems to have been a more or less completely ice-free route from Montana up the east flank of the Rockies, Mackenzies, and Richardsons to the arctic coast. Migratory birds and northward-spreading mammals may thus have had early access to the peripheral islands. The smaller group of species that persisted in Peary Land may have penetrated the archipelago somewhat later than the plants from the mainland refugia; for the

Ellesmere ice sheet must have delayed their progress. In the 4000–5000 years of the xerothermic many plants must have had time to invade the whole of the peripheral area and also penetrate into the body of the archipelago to varying degrees. The first postglacial cold period must have stopped all advances and probably eliminated some species from parts of their ranges. At present we cannot safely distinguish the effects of the first cold period from the second. It is hoped that palynology and radiocarbon studies of bog deposits in biologically richer parts of the archipelago will soon clarify the picture. Certainly some plants must have lost ground and some, at least, must have been eliminated from the northwestern Queen Elizabeths in the first cold period. Recovery during the interval between the two cold periods must have been much slower than during the xerothermic and may not have been very important, for the interval was probably little more than 1000 years and the climate little better than today, i.e. perhaps 3–4° F colder in July than during the xerothermic. The two cold periods between them, but perhaps the second one in particular, not only delayed plant migration, but certainly eliminated many species from the northwestern islands. Some of the species that I have placed in class *B* must have started as peripheral plants and become general before the end of the xerothermic. Such species were eliminated from the northwestern islands during one of the cold periods. It is also probable that most of the species in class *D* were in the northwestern islands before the end of the xerothermic.

This interpretation of the glacial and postglacial history of the northwestern Queen Elizabeth Is. makes it necessary to reassess the question of endemism and refugia in the archipelago as a whole. The distribution patterns strongly emphasize the importance of the well-known unglaciated areas of the interior of Alaska and Yukon as refugia. They also seem to show beyond reasonable doubt that Peary Land, indicated by Flint (1949) as being unglaciated, was a true refugium and not merely thinly covered with ice. The evidence for refugia within the archipelago is very unconvincing. Only three species of vascular plants appear, from their known distribution, to be currently confined to the islands: *Poa nascopieana*, known only from eastern Baffin; *Puccinellia poacea*, known only from a few stations in the Eureka Sound region of eastern Axel Heiberg and western Ellesmere (Porsild, 1955, p. 82); and *Puccinellia bruggemannii*, known from Prince Patrick, Bathurst, Cornwallis, North Devon, Somerset, and King William Is., and Spence Bay (the last station being actually on the mainland). *Poa nascopieana* comes from an area that was certainly wholly glaciated; it may be only a teratological form of another species (Polunin, 1959, p. 66) or a postglacial variant. *Puccinellia poacea* is known only from a region that is believed to have been completely and heavily glaciated. The precipitation at Eureka is admittedly very light, but all the lowlands in the region were overwhelmed by ice thrusting out from the surrounding high mountains during the last glaciation. *P. poacea* is perhaps a postglacial variant of *P. arctica*, which was nearly obliterated in the postglacial cold periods, but was able to persist in this area of relatively light snowfall and high temperature. This process of rapid spread in the xerothermic, followed by extinction from all but the most favored localities in the later cold periods, also explains much of the floristic

richness of Hazen Lake. *P. bruggemannii* has one station in the supposedly unglaciated area (Prince Patrick I.) but occurs far eastward as well as south just to the mainland. It was not described until 1955 and belongs to a genus whose species are very difficult to recognize in the field. It varies considerably in stature and habit, often appearing very like *P. angustata*. We certainly cannot yet be sure that it does not occur appreciably outside its known range. There are a few other plants, such as *Parrya arctica*, for which most of the records are from the arctic islands and relatively few from the mainland. However, such species do not necessarily indicate an island refugium. They may have been largely excluded from the Mackenzie lowlands by increasing temperatures or other factors. Moreover, some of these plants may still occur to an unrealized extent in the Ogilvie, Richardson, and eastern Mackenzie Mts. These mountains are still relatively inaccessible and are quite inadequately explored botanically.

If there was a refugium anywhere in the archipelago, Banks I. seems to be its most probable location. This region did at least evidently escape from being overwhelmed in the two postglacial cold periods; but, although it harbors some plants that do not or scarcely reach the islands elsewhere these plants are all also amply recorded on the mainland. Weather records are now available for 5 years from Sachs Harbour on southwest Banks I.; these records indicate summer temperatures scarcely higher than at Resolute and about 3° F higher than at Isachsen, emphasizing the influence of chilling winds off the Beaufort Sea. However, the interior of Banks I. evidently enjoys an appreciably sunnier summer than the outer coast (J. B. Fyles, personal communication). Nevertheless if the atmospheric circulation was not markedly different during the Wisconsin glaciation, the likelihood of much of Banks I. having become regularly snow-free in summer seems remote. Under the circumstances I find difficulty in seeing any basis for the enthusiastic suggestions of, e.g., Löve (1959), who states that in the arctic archipelago "there can be no doubt that many plants and animals...had ample opportunities even throughout the heaviest glaciations of the boreal area." It may be noted that Hultén (1937), who showed an astonishing skill in interpreting the glacial history of the Bering St. and North Pacific regions, was at a severe disadvantage in having to deal with the Canadian arctic at a time when both glaciological and botanical coverage were most inadequate. We now know that large areas which he believed to have been completely free of ice were heavily glaciated. We also know that some of the gaps in distribution that must have seemed to him merely to reflect lack of collecting are real.

My interpretation of the postglacial history of the flora of the northwestern islands indicates that most if not all of the plants have recolonized the outer islands during the last 200–300 years. As I have already shown (Savile, 1956), even in the interior of British Columbia, where the relief is very high, many plants have sustained a postglacial migration rate of 100 miles per 1000 years. In regions of low relief, rates many times as fast may be expected. Among the northwestern islands, where the sea is devoid even of shore leads for 9 months of the year and never becomes significantly open, we have, in effect, a level plain from which the islands emerge as low hills. Except during

the brief summer, hard-packed snow fills gulleys and further smooths the relief. The average annual wind speed at Isachsen is about 10 m.p.h. Although substantial calms occur in winter, they alternate with strong winds or gales from almost any direction. The minimum distance from either Bathurst or Axel Heiberg to Ellef Ringnes is about 80 miles, and that from Axel Heiberg to Amund Ringnes is 26 miles. Seeds, or parts of plants bearing seeds, must not infrequently be blown such distances in a single gale. Because of eddy action they must often be deposited at the base of a cliff or slope, where the chance of survival is good. It must further be remembered that effective wind-dispersal is not restricted in the arctic to plants with plumed seeds or fruits. In *Saxifraga* the seeds are shaken from the erect capsules only when the wind is strong enough to carry them a substantial distance, which means that they are often dispersed over the snow. It is suspected that a somewhat similar mechanism operates in some other genera. Thus there are effective means of rapid transport for many plants quite apart from the role played by animals. Among the birds and mammals that occur on Ellef Ringnes I. the Brant Goose (*Branta bernicla*) is probably the most important carrier of seeds. In spring it grazes on many overwintered plants in both wet and dry habitats. It inevitably ingests numerous seeds, the majority of which probably pass through the intestinal tract with normal or enhanced germinability, as is well known to be true of various ducks. (See summary in Ridley, 1930.) Simmons (1913) was inclined to disparage the Brant as a dispersal agent, but, after studying its spring grazing habits, I believe it to be particularly important. In these islands, where much of the wind is from the north and northwest, the Brant may well be the most important single dispersal agent, physical or biological. Another factor that contributes to rapid dispersal in the arctic is the minor biological competition. With nearly every ecological niche incompletely filled, seeds have a strong chance of establishing plants wherever they lodge.

Under the section Climate and Weather attention was drawn to the contrast between the summer climate of Peary Land and that of various parts of the archipelago. When we study the distributions of plants in classes *C*, *D*, *I*, and *J* in connection with the present climate and the lack of signs of glaciation in much of Peary Land, it seems beyond question that it was an effective refugium during the Wisconsin glaciation. What its total flora was we cannot say, for it may have supported some of the plants in several other classes as well as a few beyond the scope of this paper.

There is considerable divergence of opinion as to the temperature depression during the Pleistocene glaciations. The estimates cited by Flint (1947) range from 3 to 8° C (ca. 5 to 14° F). If, as seems probable, there was also an increase in snowfall, a considerably smaller temperature drop would have initiated glaciation in many mountainous regions as well as leaving great lowland areas snow-covered. Once considerable land remained snow-covered, glaciation in adjoining areas would be accelerated. If we suppose that, throughout the nearctic, there was a general drop of 4° F, an increase in snowfall of 10 inches (i.e. approximately a 50% increase for some of the drier areas), and no striking change in the prevailing winds, we see that summer in Peary

Land, with June, July, and August means of 33, 39, and 35° F, would be comparable with that today at Resolute—not quite as warm as Eureka, but slightly warmer than Alert, Isachsen, and Mould Bay. In other words Peary Land might still harbor 60 or more species of higher plants. The fate of the Canadian archipelago under such conditions would have been very different. Permanent snowfields would quickly have covered the outer northwestern islands. Every extension of these snowfields would have reduced the warming of winds flowing across them and hastened the formation of permanent snow cover on the inner islands. Concurrently the mountain glaciers on Axel Heiberg, Ellesmere, Devon, and Baffin Is. would have been increasing and would eventually have spread out to meet the snowfields of the western islands. I make no claim that the Pleistocene glaciations were produced by such a modest temperature drop; but it does seem probable that during the Wisconsin glaciation the fall in temperature did not greatly exceed 4° F, for it is unlikely that Peary Land would otherwise have remained free from permanent snow. A possible alternative explanation is that nearly all the wind reached Peary Land from over the main Greenland icecap. As the icecap grew the passing air would lose progressively more moisture, and, picking up the latent heat of condensation and freezing, would reach Peary Land even more strongly warmed than today.

Holmen (1957) was dubious about Peary Land having been ice-free during the Wisconsin glaciation, despite its present climate and its flora of 97 species of vascular plants. His doubts were evidently due largely to the indications of marine submergence; but such submergence may have resulted from interglacial marine transgression rather than depression of the land by ice.

During a discussion of this interpretation of the Wisconsin and postglacial history of the archipelago, the question arose as to whether any other regions had been subject to the light but general snow cover postulated for the northwestern Queen Elizabeth Is. At first it seemed probable that very similar conditions must have operated in other arctic areas that had low relief but were close to the Arctic Ocean. Shortly afterwards, however, I chanced upon a diagram by Crary (1960) showing lines of equal heat, in kilocalories above 0° C per $cm^2$, for the Arctic Ocean. Crary shows a maximum of 120 kcal from approximately northern Spitzbergen to northern Severnaya Zemlya. The values drop off rapidly toward the North Pole where the figure is ca. 25 kcal, with the lines closer toward the nearctic than palearctic coasts. The values diminish more slowly beyond the pole, but do still decrease until a minimum value of 12 kcal is reached at a line nearly along the edge of the continental shelf off the northwestern Queen Elizabeth Is. This distribution of heat values seems to go far toward answering our question as well as helping to explain both the temperature and plant distribution patterns in the archipelago. With such a distribution of heat in the Arctic Ocean and with an appreciable predominance of north and northwest (onshore) winds, the bleakness of the summers in these outer islands is inevitable. Whether the extension of the northwestern gap to Somerset I. reflects the present climatic pattern or the pattern in the last climatic deterioration can scarcely be answered from the climatic data currently available.

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# THE GENUS ASCODESMIS[1]

WALTER OBRIST[2]


## Abstract

A study of the physiological, cytological, and morphological characteristics of four species of the genus *Ascodesmis* has been made. The genus represents a highly specialized coprophilous group of the operculate discomycetes, probably belonging to the Humariaceae. A new specific name (*Ascodesmis sphaerospora* nom. nov.) and a new species (*Ascodesmis macrospora* sp. nov.) are proposed.


## Introduction

*Ascodesmis* van Tieghem is a genus of the operculate discomycetes characterized by the lack of an excipulum. It was, therefore, considered to represent a primitive form of the ascomycetes. Cytological investigations carried out by Claussen (1905) and Swingle (1934) appeared to prove this hypothesis. But Le Gal (1949) claims, on account of the complicated spore structure, that the genus is more likely to be a higher but regressive form. This author also clarified the taxonomic position of two species, which had been misinterpreted.

While investigating coprophilous ascomycetes on dung of brazilian animals, I succeeded in isolating several strains of *Ascodesmis* species. A study of these isolates has been made in an attempt to further clarify the phylogenetic position and species concept of the genus.

## Spore Germination

Discharged ascospores of any species of *Ascodesmis* will readily start germinating within a few hours in a moist environment. Immersed in any liquid, however, the spores do not germinate. Dried spores can be revived even after a period of many years. Three-year-old spores of *Ascodesmis sphaerospora* did germinate in our experiment.

Before forming the germ tube, the spore swells considerably and the spore wall turns brighter. The average diameter of spores of *A. sphaerospora* that had just started to form a germ tube (i.e. 6–12 hours after discharge) was 14–15 $\mu$, compared to 10–11.5 $\mu$ at the time of discharge. Each spore produces one, rarely two, germ tubes, which grow fast and soon become septate.

The branching and further growth of the hyphae is well described by Claussen (1905).

## Cultural Studies

All described species of *Ascodesmis* are coprophilous and have been found on dung of herbivorous and carnivorous animals. In cultural studies dung decoction agar has been used exclusively by previous workers (Claussen 1905, Swingle 1934). An attempt has therefore been made to grow the species in

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better-defined media. The four species *A. nigricans*, *A. sphaerospora*, *A. macrospora*, and *A. porcina* all grow well on various artificial media and do not show any difference from each other regarding both vegetative growth and production of ascocarps in any one medium. Of the numerous media tested, however, two groups can readily be distinguished regarding the abundance of growth and fruiting of *Ascodesmis* species:

1. Plain agar, malt agar, peptone agar, peptone – malt agar, V-8 agar. Fast mycelial growth in and on surface of medium, poor fruit body production.

2. Modified Leonian's (peptone, maltose, malt extract, yeast extract), soluble starch – yeast extract – agar. Fast growth and abundant aerial mycelium, fruiting very plentifully.

The last-mentioned medium proved most satisfactory and its formula is therefore given here: soluble starch, 10.0 g; yeast extract, 5.0 g; $KH_2PO_4$, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; agar, 20.0 g; tap water, 1000 ml.

In mixed cultures in which *Ascodesmis* is grown with bacteria isolated from dung, production of apothecia is increased, whereas mycelial growth is not affected. The above-mentioned soluble starch – yeast extract medium was used, and unidentified dung bacteria were grown in petri dishes in small colonies about three days before inoculation of an *Ascodesmis* species. The fungus mycelium spread rapidly from a central point. Abundant but scattered production of fruit bodies started after 1–2 days and mature ascospores appeared after 3–6 days. On the bacterial colonies, however, apothecia appeared densely clustered and mature spores were produced about one day earlier.

Similar experiments were made with liquid media in tubes and strips of filter paper soaked in liquids.

Three kinds of liquid media were used:

A. Minerals, peptone, malt extract, distilled water.

B. A + yeast extract.

C. Dung bacteria grown for 3 days in liquid B were autoclaved.

Inoculations of *A. porcina* grew slowly in the liquids but rapidly on the filter paper strips above the liquids. The results are shown in Table I.

TABLE I

Ascospore production in *A. porcina* in liquid media

| | Days required for production of mature spores | | |
|---|---|---|---|
| | A | B | C |
| Liquid medium | — | 20–24 | 12–15 |
| Liquid medium + filter paper strips | 25–38 | 5–7 | 4–5 |

It seems from these experiments that chemical factors stimulating apothecial development are supplied by bacteria in the natural habitat and that yeast extract is the most suitable, although not as efficient, substitute. Solid media are superior to liquids.

In all four species studied the production of fruit bodies in pure cultures slowly decreases after many transfers. There is an increase in the growth of aerial mycelium and most apothecia are formed immersed in the medium.

The culture of *A. nigricans* isolated by Le Gal (1949) has almost ceased to produce fruit bodies on agar media. It can be revived, however, if it is grown on sterilized dung together with bacteria.

Strains of the four *Ascodesmis* species studied are therefore better maintained in mixed cultures with bacteria isolated from dung. Production of ascocarps in such cultures remained abundant after many transfers.

Light is not required for either formation or maturation of ascospores. Monospore cultures grown without light in a thermostat at 20° C produced mature spores with normal germinating capacity. The time between inoculation and discharge of mature ascospores, however, is 1–3 days longer than in a control culture under daylight.

Similar experiments were made in a group of coprophilous pyrenomycetes, the Sordariaceae, by Moreau (1953). Whereas the Sordariaceae have almost identical nutrient requirements and are also stimulated by other microorganisms, they differ from *Ascodesmis* in that they require light for fruiting.

## Cytology and Genetics

Because of the lack of an excipulum, *Ascodesmis* species seem to be very suitable for cytological work. Van Tieghem (1876) and Zukal (1886) did not observe any sexual stages. Dangeard (1903, 1907) first saw pairs of coiled hyphae growing out of short side branches but claimed that there was no fusion between these structures, the only sexual fusion of nuclei taking place in the young asci. Claussen (1905) and Swingle (1934), however, observed nuclear fusions both between the coiled hyphal pairs (ascogonium and antheridium) and in the young asci. Since it is very difficult to demonstrate such fusions with convincing evidence because (1) all cells are multinucleate and (2) the coiled hyphal pairs occur in dense clusters in which cell walls seem to disintegrate, this problem remains unsolved.

A genetical study would probably be a better approach to these problems. An attempt was made to obtain mutant strains. Monospore cultures of *A. sphaerospora* and *A. macrospora* were subjected to irradiation with ultraviolet light. Exposures of 3–10 hours failed to induce any morphological mutation.

Hyphal fusions are quite frequent in all species studied. Such fusions seem to stimulate ascocarp production. In order to investigate the occurrence of interspecific anastomosis, all possible combinations of strains of two different species were mated in petri-dish cultures. In each case the mycelia grew together without any sign of inhibition. The analysis of fruit-bodies along the borderline always showed that the spores of all asci in any one apothecium belonged to either one or other of the two species mated. No intermediate or unidentified spores were observed. The species as described in this paper, therefore, seem to be genetically distinct and non-hybridizing. This problem, however, deserves a more extensive study.

## Asci and Spores

Shape, size, and structure of the asci are almost uniform in the genus *Ascodesmis*. The shape is very broadly clavate, the apical structure is typically of the operculate type of the discomycetes, and before discharging the spores, the asci swell considerably and project from the hymenium.

The spores are crowded in a gelatinous mass and are all discharged together in a group. In a culture of *A. macrospora*, a maximum vertical discharge distance of 20 cm has been measured. The asci bow towards the light. Under cultural conditions, the asci shoot their spores when the cover is removed. A slight air current brings about the rupture of the operculum of swollen asci. In many asci, however, the turgor pressure breaks down before the operculum is opened and the spores remain in the ascus. In each species, the asci mature successively. In this manner, spores are released from one apothecium over a period of many hours to a few days if conditions are favorable.

The spore number is usually eight, but numbers of four and five have been observed especially in cultures on dung and on media of group 1. Anomalous small and dark spores occur in numbers of one to three per ascus in *A. sphaerospora* and *A. macrospora* (Fig. 3). In rich media (group 2) these anomalies are less frequent. Monospore cultures of anomalous spores have not been made, but they are likely not to be capable of germination.

The spore features seem to be the only differentiating characters between the species. Shape, size, and markings of the spores are quite typical in each specimen or culture, but if many collections are examined, one is confused because of the considerable variability. Shape and size can be accurately measured (Fig. 1); the different types of spore markings, however, are difficult to separate. There is a general tendency towards reticulate markings, *A. nigricans* showing the least amount of reticulation and *A. sphaerospora* the most perfect pattern.

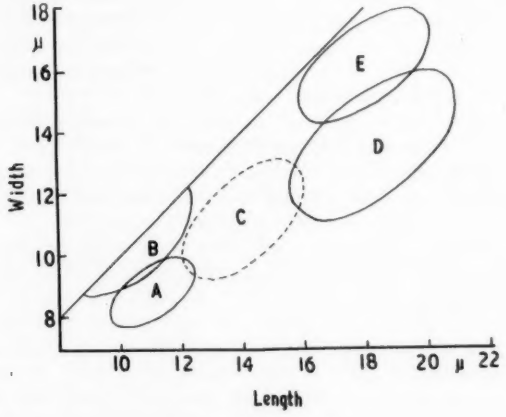



FIG. 1. Spore measurements in artificial culture.
(A) *Ascodesmis nigricans* and *Ascodesmis porcina*,
(B) *Ascodesmis sphaerospora*,
(C) *Ascodesmis microscopica* (measurements from Le Gal 1949),
(D) *Ascodesmis macrospora*,
(E) strain TRTC 35537, *Ascodesmis macrospora*, in natural habitat.

Le Gal (1949) recommends the use of cotton blue for staining the spore markings. This stain is useful for the light brown spores of *A. nigricans* and

for immature spores. The perisporium, a hyaline sheath surrounding the spores of *A. microscopica* and *A. macrospora*, will stain slightly greyish. The dark brown reticulations of fully mature spores of *A. sphaerospora* and *A. macrospora*, however, are not stained by cotton blue.

## Description of the Genus *Ascodesmis*

Mycelium hyaline, septate, multinucleate, branching monopodially, growing circularly on agar media, spreading rapidly. No conidia or spermatia produced. Ascocarp initials consisting of one to three (mostly two) short hyphal side branches, branching dichotomously and producing more slender branches which coil around each other in pairs.

Apothecia minute, globose, scattered or gregarious, hyaline, without excipulum, consisting of a cluster of asci and surrounding paraphyses springing from a clump of basal cells.

Asci broadly clavate, ovoid or elliptical, fasciculate, maturing successively, rounded above, opening by a somewhat obliquely attached operculum, thin-walled, contracted below into a short stipe, swelling before spore discharge, not stained by iodine, eight- (exceptionally four- to five-) spored. Paraphyses numerous, stout, enlarged above, septate, unbranched, originating from the lower cells of the basal cell mass.

Spores one-celled, spherical to ellipsoid, hyaline when young, becoming brown at maturity, two-seriate or irregularly arranged, crowded together in upper part of ascus before discharge, shot off together, covered with dark brown markings in the form of spines, ridges, or reticulations, with or without hyaline perisporium.

Type species: *Ascodesmis nigricans* van Tieghem.

### Key to the Species

1. Spores partially or entirely surrounded by a hyaline outer membrane, the perisporium; longer than 12.5 $\mu$ ..... 2
1. Spores without perisporium; length up to 12.5 $\mu$ ..... 3
2. Spores 12.5–16 $\mu$ long, completely covered by perisporium ..... *A. microscopica*
2. Spores 16–21 $\mu$ long, partially covered by perisporium ..... *A. macrospora*
3. Spores globose or subglobose (difference between length and width less than 1.5 $\mu$), with reticulate markings ..... *A. sphaerospora*
3. Spores ellipsoid, never reticulate ..... 4
4. Spore markings consisting of spines and short angled ridges ..... *A. nigricans*
4. Spore markings consisting of spines and one longitudinal ridge ..... *A. porcina*

### *Ascodesmis nigricans* van Tieghem (Fig. 10)

SYNONYMS: ? *Ascodesmis echinulata* Bainier. Bull. Soc. Myc. Fr. 23:137, 1907.
*Ascodesmis nigricans* van Tieghem. Bull. Soc. Bot. Fr. 23:275, 1876.

HABITAT: Dung of dog, sheep, goat, pig, fox, peccary, jaguar, ocelot.

NEOTYPE: Culture No. 100, Laboratoire de Cryptogamie du Museum Nationale d'Histoire Naturelle, Paris, Le Gal (1949).

SPECIMENS EXAMINED: Neotype. On fox dung, Nashville, York Co., Ontario, May 20, 1956, R. F. Cain (TRTC 32679). On dog dung, Nashville, York Co., Ontario, Dec. 5, 1959, R. F. Cain (TRTC 35292). On dung of jaguar (TRTC 35536), Ocelot (TRTC 35599), and peccary (TRTC 34759), Recife, Brazil, June 27, 1957, A. C. Batista. On dung of carnivorous animal, Colfax, La., U.S.A., Aug. 26, 1960, R. F. Cain (TRTC 36466).

Apothecia 80–180 $\mu$. Asci 42–57×18–25 $\mu$. Paraphyses 3–5 $\mu$, slightly enlarged above (up to 5.5 $\mu$).

Spores ellipsoid, without markings 9.5–11.5×8–10 μ, with markings 11.5–12.5×9–10.5 μ, difference between length and width more than 1.5 μ, pale brown at maturity. Sculpturing consisting of spines, wart-like projections, short ridges, or irregularly angled ridges (see Le Gal 1949 and Fig. 10).

According to Le Gal (1949) van Tieghem's type specimen is lacking. It is suggested, therefore, that Le Gal's specimen be designated the neotype of the species.

*Ascodesmis sphaerospora* nom. nov. (Figs. 2–9)

SYNONYM: *Ascodesmis microscopica* (Crouan) Seaver. Mycologia, 8:3, 1916.
non *Ascodesmis microscopica* (Crouan) Le Gal. Rev. Myc. 14:93, 1949.

HABITAT: Dung of various animals (dog, wild boar, jaguar).

TYPE: Culture isolated from sewage trickling filter; Dayton, Montgomery Co., Ohio, June 1955, Wm. B. Cooke (TRTC 32092).

SPECIMENS EXAMINED: Type. On dog dung, Cambridge, Mass., 1891, W. C. Sturgis (Herb. N.Y. Botanical Garden). On dung of raccoon-dog, Bronx Zoological Garden, N.Y., Nov. 1915, F. J. Seaver, type of *Ascodesmis microscopica* (Crouan) Seaver (Herb. N.Y. Botanical Garden). On dung of wild boar, Tamsel, Brandenburg, Germany, Febr. 22, 1935, P. Vogel, developed in laboratory, Toronto, Ont., Dec. 6, 1935, R. F. Cain (TRTC 34710). On dung of jaguar (TRTC 35535) and ocelot (TRTC 35598), Recife, Brazil, June 27, 1957, A. C. Batista. On dog dung, Nashville, York Co., Ont., Dec. 5, 1959, R. F. Cain (TRTC 35293). On dung of carnivorous animal, Colfax, La., U.S.A., Aug. 26, 1960, R. F. Cain (TRTC 36467).

Mycelia hyalina, tenua, sine conidiis et spermatiis. Ascogonia in ramis lateralibus disposita. Apothecia minuta, subglobosa vel hemisphaerica, gregaria, 80–150 μ diam., superficialia, hyalina, ex fasciculis (3)–10–18 ascorum et numerosorum paraphysium circumdatorum ex gleba basali crescentibus composita. Asci clavati vel elliptici, 55–80×24–33 μ, fasciculati, apice rotundati, in stipitem brevem contracti, operculati, unitunicati, tunica tenui (solum in parte basali densata) praediti, octospori, raro 4–5-spori, in ordinem maturescents. Paraphyses numerosi, hyalini, septati, 3.5–5 μ diam.; in apice ad 6 μ densati, circa ascos dispositi.

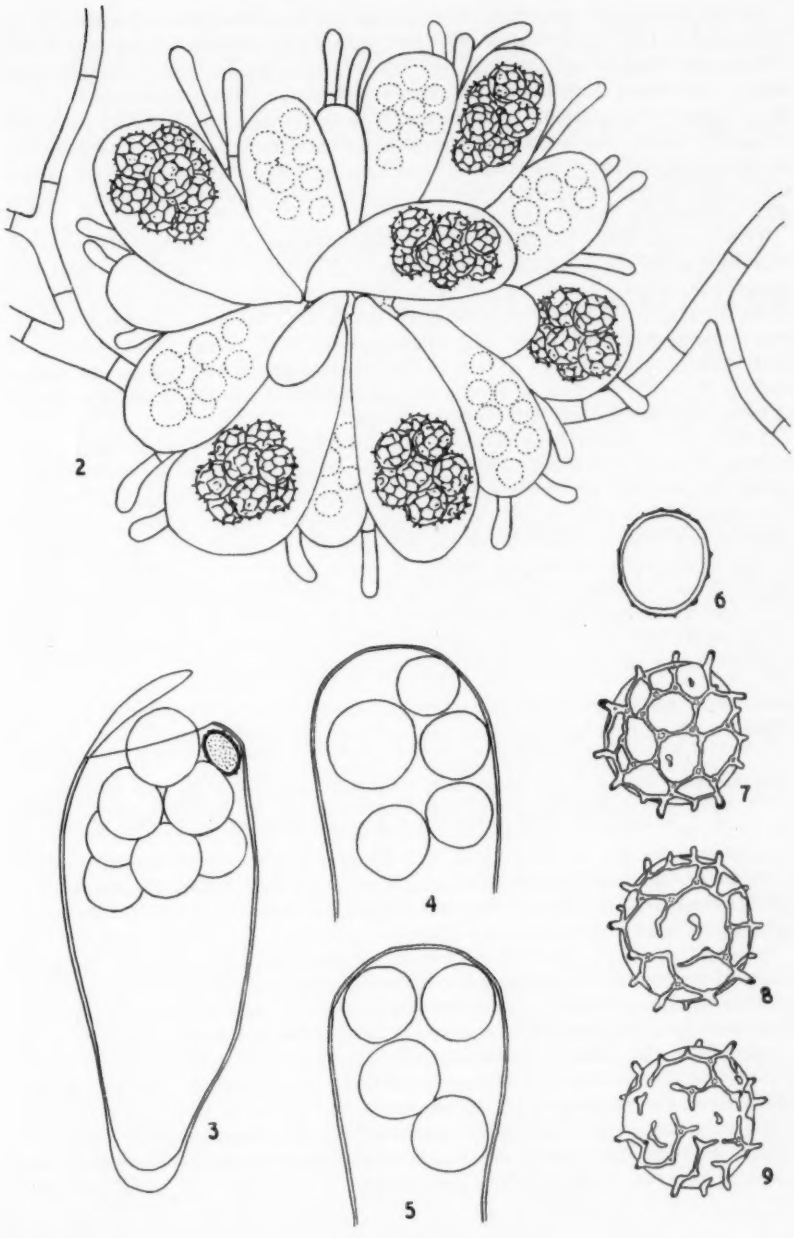
Ascosporae unicellulares, globosae vel ellipticae, cum spinis 11–15×10.5–14 μ, sine spinis 8.5–12.5×8.5–12 μ, cohaerentes, primum hyalinae, maturitate atrofuscae, areolatae, aculeatae, reticulatae, episporio tenui.

Apothecia minute, hemisphaerical to subglobose, 80–200 μ in diameter, scattered or gregarious, hyaline, superficial, without excipulum, with many asci surrounded by paraphyses.

Asci broadly clavate, oblong or ovoid, 55–80×24–33 μ, fasciculate, rounded above, opening by a slightly obliquely attached operculum, thin-walled, contracted below into a short, thick-walled stipe, eight-, exceptionally four- or five-spored, maturing successively, 10–18 (sometimes less) visible at one time, three–eight with mature spores. Before spore discharge, the asci swell to a length of up to 92 μ, the operculum is then opened and the spores shot off altogether through internal pressure.

Paraphyses numerous, stout, 3.5–5 μ in diameter, gradually enlarged above (up to 6 μ), in culture sometimes forming globose endings of 6–8 μ, septate, unbranched, slightly longer than the asci.

FIGS. 2–9. *Ascodesmis sphaerospora*. Fig. 2. Apothecium seen from above (×500). Fig. 3. Ascus with one anomalous small spore (×1000). Figs. 4 and 5. Asci with fewer than eight ascospores (×1000). Fig. 6. Young ascospore in optical section (×2000). Figs. 7–9. Mature ascospores (×2000).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Spores one-celled, spherical to ellipsoid, with markings 11–15×10.5–14 μ, without 8.5–12.5×8.5–12 μ, if elliptical, difference between length and width always less than 1.5 μ, biseriate or crowded together, first hyaline, then pale brown, becoming dark brown at maturity. Sculpturing consisting of net-like, dark brown reticulations (meshes reaching a diameter of 2–3.5 μ) and (or) irregular ridges, the reticulations and ridges projecting about the periphery of the spore and forming spines (length up to 2 μ) at their junctions. Spore wall thick in young spores, thin in mature spores. No perisporium. Cotton blue stains only immature spores.

This fungus has apparently been misinterpreted by Seaver (1916). The reinvestigation of *Ascodesmis microscopica* (Crouan) by Le Gal (1949) has shown that Seaver's species can be neither *A. nigricans* nor *A. microscopica*. Unfortunately, in many mycological textbooks Seaver's combination *A. microscopica* has been used (e.g. Alexoupoulos 1952, Gray 1959, Wolf and Wolf 1947). *A. nigricans* differs in having non-reticulate spores, *A. microscopica* (Crouan) Le Gal in producing a perisporium. In addition, the spores of these two species are typically ellipsoid.

*Ascodesmis sphaerospora* shows a considerable variation in number, size, and shape of the ascospores. If growing on dung the spores are spherical or nearly so, whereas on artificial agar media they tend to be more elliptical. Four-spored asci are rather rare, the five-spored asci occurring more frequently, but both of these anomalies have not been observed in agar cultures. Single, imperfectly developed spores in eight-spored asci, however, can be found on any medium quite often, these spores being small (5–7.5 μ), very dark and with reduced, indistinct sculpturing (see Fig. 3). The spore sculpturing may vary from regular reticulations to irregular, angled ridges. The relatively long spines are a constant feature in this species.

*Ascodesmis porcina* Seaver (Fig. 11)

HABITAT: Dung of pig, peccary, horse.

TYPE: On pig dung from Puerto Rico, grown in the laboratories of the New York Botanical Garden, Jan. 1915, F. J. Seaver (Herb. N.Y., Botanical Garden).

SPECIMENS EXAMINED: Type. On pig dung from New Jersey, Nov. 26, 1915, F. J. Seaver (Herb. N.Y. Botanical Garden). On dung of peccary, Recife, Brazil, June 27, 1957, A. C. Batista, developed in laboratory, Toronto, Aug. 17, 1960 (TRTC 36308).

Apothecia 80–200 μ. Asci 55–65×22–28 μ, 7–18 visible at one time, two to seven of them with mature spores, swelling before spore discharge to a length of up to 75 μ. Paraphyses not numerous, stout, 3.5–5 μ in diameter, slightly enlarged above (up to 5.5 μ).

Spores with markings 12.5–15×10–11 μ, without 10–12.5×8–9 μ, pale brown at maturity. Sculpturing consisting of rounded, wart-like projections and short interrupted ridges, usually with one conspicuous simple or branched ridge extending longitudinally across one side of the spore (see Fig. 11).

Since Seaver's (1916) original description, this species has been reported only by Meyer and Meyer (1949) on horse dung from Panama and by Gunnell (1960) from Great Britain. It is, therefore, likely to be widely distributed throughout the world. The spore markings are of a very peculiar type and readily separate the species from any other. Size, shape, and color of the spores agree very closely with *A. nigricans*.

*Ascodesmis microscopica* (Crouan) Le Gal

SYNONYMS: *Ascobolus microscopicus* Crouan. Ann. Sc. Nat. 4. sér. Bd. 7:175, 1857.
*Boudiera microscopica* (Crouan) Cooke. Grevillea, 6:76, 1877.
*Ascodesmis microscopica* (Crouan) Le Gal. Rev. Mycol. 14:93, 1949.
*Boudiera Claussenii* Hennings. Beibl. Hedwigia, 42:182, 1903.

HABITAT: Dung of various kinds.

TYPE: *Ascobolus microscopicus* Crouan, "sur l'Album graecum, prés Brest", in herbarium Montagne, Museum Nationale d'Histoire Naturelle, Paris.

Apothecia 200–300 μ. Asci broadly clavate, 63–80×26–33 μ. Spores subglobose to ellipsoid, without markings 12.5–16×9.5–13.25 μ, with markings 14–18.5×11–15 μ, at maturity dark brown, sculpturing consisting of irregular reticulations, short ridges and spines, surrounded by a perisporium, which is stained by cotton blue.

The type of this species has been reinvestigated and described by Le Gal (1949).

*Ascodesmis macrospora* sp. nov. (Fig. 12)

HABITAT: Dung of mountain lion and jaguar.

TYPE: TRTC 35512. Culture isolated from dung of mountain lion (Felis concolor), Recife, Brazil, June 27, 1957, A. C. Batista, developed in moist chamber, Toronto, Jan. 25, 1960.

OTHER SPECIMENS EXAMINED: On dung of jaguar (TRTC 35537) and Raposa do campo (Cerdocyon thous) (TRTC 35596), Recife, Brazil, June 27, 1957, A. C. Batista, developed in laboratory, Toronto, 1960.

Mycelia hyalina, tenua, sine conidiis et spermatiis. Ascogonia in ramis lateralibus disposita.

Apothecia minuta, subglobosa vel hemisphaerica, gregaria, 90–200 μ, diam., superficialia, primum hyalina, maturitate atrofusca, ex fasciculis (5) 10–20 ascorum et numerosorum paraphysorum exgleba basali crescentibus composita. Asci clavati vel elliptici, 72–90×25–35 μ, fasciculati, apice roturdati, in stipitem brevem contracti, operculati, unitunicati, tunica tenui (in parte basali densata) praediti, octospori (raro 4–5-spori), in ordinem maturescentes. Paraphyses numerosi, hyalini, septati, 4–5.5 μ diam., circa ascos dispositi.

Ascosporae unicellulares, ellipticae, cum spinis 18.5–23×14–16.5 μ, sine spinis 16–21×12–15.5 μ, cohaerentes, irregulariter dispositae, primum hyalinae, maturitate atrofuscae, reticulatae, aculeatae, episporio tenui et in parte perisporio hyalini circumventae.

Mycelium in culture hyaline, branching monopodially, spreading rapidly. No conidia or spermatia produced. Ascogonia and antheridia as side branches growing side by side on mycelium, forming dichotomously branching structures, the ends of which coil around each other in pairs.

Apothecia minute, hemisphaerical to subglobose, 90–200 μ, in diameter, scattered, first hyaline, becoming dark brown at maturity, superficial (in culture also immersed), without excipulum, the asci surrounded by paraphyses. Asci broadly clavate, oblong, elliptical or ovoid, 72–90×25–35 μ, fasciculate, rounded above, opening by a slightly obliquely attached operculum, thin-walled, contracted below into a short, thick-walled stipe, eight- (exceptionally four- to five-) spored, maturing successively, 10–20 visible at one time, three to nine of them with mature spores, swelling before spore discharge to a length of up to 110 μ.

Paraphyses numerous, stout, 4–5.5 $\mu$ in diameter, very slightly enlarged above (up to 6 $\mu$), septate, unbranched, growing up earlier than the asci.

Spores one-celled, elliptical, with markings 18.5–23×14–16.5 $\mu$, without markings 16–21×12–15.5 $\mu$, crowded together, first hyaline, then pale brown, becoming dark brown at maturity. Sculpturing consisting of irregular or interrupted, net-like reticulations (meshes with a diameter of 3–7 $\mu$), short ridges, and scattered spines. Parts of the spore surface covered by a hyaline, gelatinous perisporium. Spores discharged at once through opening of operculum (Fig. 12).

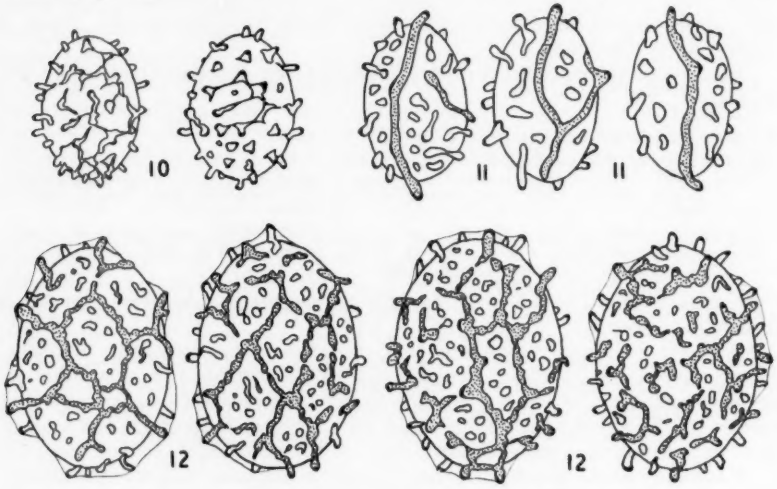


FIG. 10. Ascospores of *Ascodesmis nigricans* (×2000).
FIG. 11. Ascospores of *Ascodesmis porcina* (×2000).
FIG. 12. Ascospores of *Ascodesmis macrospora* (×2000).

This species resembles *A. microscopica*, but the spores are larger and only partially covered by a hyaline perisporium.

## Conclusions

1. The species studied show almost identical physiological and cytological properties. The genus is highly specialized in its coprophilous habitat. Certain growth factors required are probably supplied by other microorganisms, particularly bacteria.

2. *A. nigricans*, *A. sphaerospora*, and *A. macrospora* are homothallic. All species studied have multinucleate cells and the ascocarps develop in the same manner as described by earlier workers.

3. Morphological characters useful for separation of species are size, shape, structure, and markings of the spores.

4. *Ascodesmis* species investigated under the name *A. nigricans* by Dangeard (1903, 1907) and Claussen (1905, 1912) is probably *A. microscopica*, while that investigated by Zukal (1886) and Swingle (1934) is probably *A. sphaerospora*.

5. On account of the physiological specialization and the complicated spore structure it is believed that the genus *Ascodesmis* represents a high evolutionary level rather than a primitive form of the operculate discomycetes (a hypothesis established by Le Gal, 1949).

6. The relationship of the genus can not be determined with our present knowledge. The spore structure suggests a relationship to *Boudiera* and *Lamprospora*. Therefore, the genus *Ascodesmis* may be tentatively placed in the family Humariaceae, tribe Humarieae sensu Le Gal (1947). The family Pyronemaceae sensu Grelet (1945) and the tribe Pseudo-Ascoboleae sensu Le Gal (1947) are probably artificial groupings.

## Acknowledgments

The author wishes to express his sincere gratitude to Dr. R. F. Cain for his interest and advice. Thanks are also due to Prof. A. C. Batista, University of Recife, for the numerous collections of dung from Brazil. Grateful acknowledgment is made to Mme Le Gal, Paris, France, and the Centralbureau voor Schimmelcultures, Baarn, Holland, for the culture of *Ascodesmis nigricans*.

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# THE USE OF AMMONIUM SOURCE OF NITROGEN IN THE METABOLISM OF ASPERGILLUS NIDULANS EIDAM[1]

KAISER NAGUIB[2] AND KAMEL SADDIK


## Abstract

*Aspergillus nidulans* was grown as surface cultures on three ammonium salts, namely: ammonium chloride, ammonium carbonate, and ammonium nitrate in a basal medium favorable for fat formation. It was also grown on sodium nitrate for comparison. Growth, uptake of sugar and nitrogen, fat and protein syntheses, and pH change in the culture media were all followed over an incubation period of 20 days at 25° C.

Growth on ammonium chloride was suppressed after a short period, when the pH of the medium dropped to a very low value. Mycelial felts had high protein contents but insignificant amounts of fat. In ammonium carbonate cultures, fungal growth continued yielding heavy mycelial felts with high fat content. But nitrogen utilization ceased while an ample supply was still present in the culture medium leading to low protein content in the mycelial felts. This could not be attributed to any drastic change in the pH of the culture medium; the pH changed only within reasonable limits.

Growth was best on ammonium nitrate. Mycelial felts contained high protein and high fat. The change in pH of the medium, during the early period of incubation, indicated preferential absorption of ammonium to nitrate ions. Growth on sodium nitrate started by a lag period, but soon became active and gave rise to a heavy mycelium.

The present findings also showed that nitrate nitrogen was more conducive to protein synthesis by *Aspergillus nidulans* than was ammonium nitrogen. Fat formation, on the other hand, was lower on sodium nitrate than on ammonium carbonate or ammonium nitrate.


## Introduction

In a previous communication (Naguib and Saddik (16)), it was reported that *Aspergillus nidulans* could grow and flourish on sodium nitrate and asparagine but developed very poor growth on ammonium chloride. Synthesis on ammonium chloride of proteins, carbohydrates, and fats almost stopped after 5 days. It was suggested that this behavior may be attributed to the large drop in the pH value of the medium through preferential absorption of ammonium ions to chloride ions. It was also concluded that the attitude of *Aspergillus nidulans* towards ammonium nitrogen cannot be clarified without eliminating the effect of limiting factors such as the change in the pH value of the culture media.

It has been reported by several investigators such as Talley and Blank (24), Brian and co-workers (3), Pai (17), Craseman (4), and Apparoa (1) that fungi may develop poor growth on ammonium nitrogen. In many cases, as with *Karlingia rosea* (Haskins and Weston (8)), *Myrothecium verrucaria* (Darby and Mandels (5)), *Diplocarpon rosae* (Shirakawa (20)), and *Choanephora cucurbitarum* (Barnett and Lilly (2)), it has been shown that the pH value markedly drops when these fungi are grown in media containing ammonium salts of strong acids such as chloride, sulphate, or nitrate, consequent

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upon utilization of the ammonium ion, and that the poor growth may be attributed to this drop in the pH value of the culture medium.

There is also some confusion about whether the inability of a fungus to utilize ammonium nitrogen is a true character of the fungus or is brought about by external factors. In this connection, it is interesting to mention that Craseman (4) concluded that the very poor growth of some chytrids on inorganic nitrogen does not indicate that they are unable to utilize it and that the nitrogen source is not the limiting factor.

In this investigation, an ammonium salt of a weak acid, namely, ammonium carbonate, was used instead of ammonium chloride, so that preferential absorption of the ammonium ion would not cause a drastic change in the pH value of the culture medium.

It was further found advisable to compare the behavior of the fungus on ammonium carbonate with that on ammonium nitrate and sodium nitrate; variation in behavior of different fungi towards ammonium and nitrate nitrogen is still worthy of investigation.

## Materials and Methods

The fungus used in the present investigation was *Aspergillus nidulans* Eidam obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland. It was subcultured every fortnight on Dox's agar.

*Composition of Media and Procedure*

The fungus was grown as surface cultures on a basal medium composed as follows (g/100 ml): glucose, 17; sodium dihydrogen phosphate, 0.73; potassium sulphate, 0.022; magnesium sulphate, 0.500; zinc sulphate, 0.005; and ferric chloride, 0.016. The nitrogen source was then added at a concentration of 105 mg of nitrogen/100 ml of medium in the form of ammonium chloride, ammonium carbonate, ammonium nitrate, or sodium nitrate. The pH was adjusted at 6.8. The experiment was further conducted in the same way as previously described (Naguib and Saddik (16)). Analyses were carried out after 3, 5, 7, 9, 12, 16, and 20 days.

*Methods of Analysis*

Estimations of residual sugar and total nitrogen in the culture media and of protein and fat in the mycelial felts were carried out by procedures referred to in a previous communication (Naguib (15)).

Determination of the pH value of the culture media at the stated intervals was performed by the standard B.D.H. capillators.

## Results

*Sugar Uptake*

Results plotted in Fig. 1, show that absorption of sugar continued quite steadily in both sodium and ammonium nitrate media, though at a higher rate in the latter than in the former, until sugar almost completely disappeared in both media after 12 days.

On ammonium chloride, absorption stopped while a large amount of sugar was still present in the culture medium. On ammonium carbonate, absorption

was steady at first but later declined and completely stopped before complete exhaustion of the sugar.

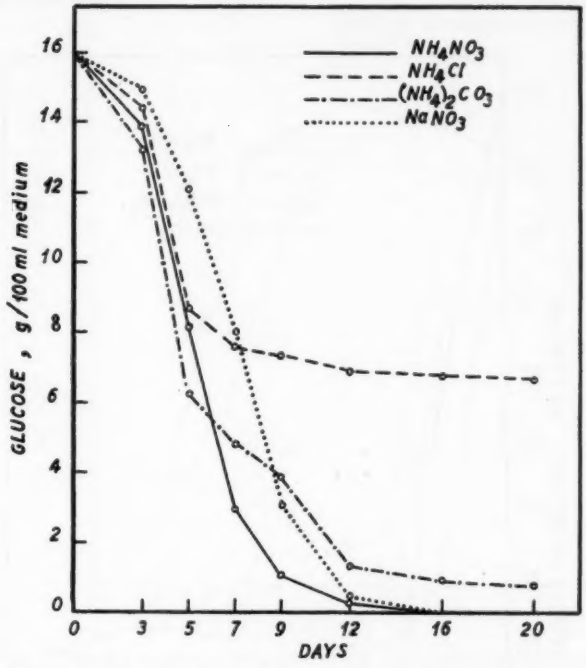


FIG. 1. The sugar concentration of the culture media (expressed as grams glucose) during the incubation period.

*Nitrogen Uptake*

It can easily be noted from Fig. 2 that nitrogen was steadily absorbed from the four media tested during the early incubation period. But, while absorption continued on sodium nitrate and ammonium nitrate until only a small amount was left, the ammonium chloride and ammonium carbonate media still contained large amounts of nitrogen when absorption stopped.

It is interesting to note also that nitrogen was released from the mycelial felts into the external media in the four sets of cultures, but the biggest release was noted in the ammonium chloride cultures.

*Dry Weights of the Mycelial Felts*

With the exception of an initial lag period, growth as indicated by the dry weight increase (Fig. 3) was most rapid on ammonium nitrate. On sodium nitrate, growth was very slow at first but later almost reached the level of the ammonium nitrate cultures. In ammonium carbonate cultures, growth started at a high rate which later declined slightly so that the dry weights

became less than those of felts fed with either ammonium or sodium nitrate. On these three media, maximum dry weight was reached on the 12th day. On ammonium chloride, growth continued at a moderate rate for 5 days only and then completely stopped.

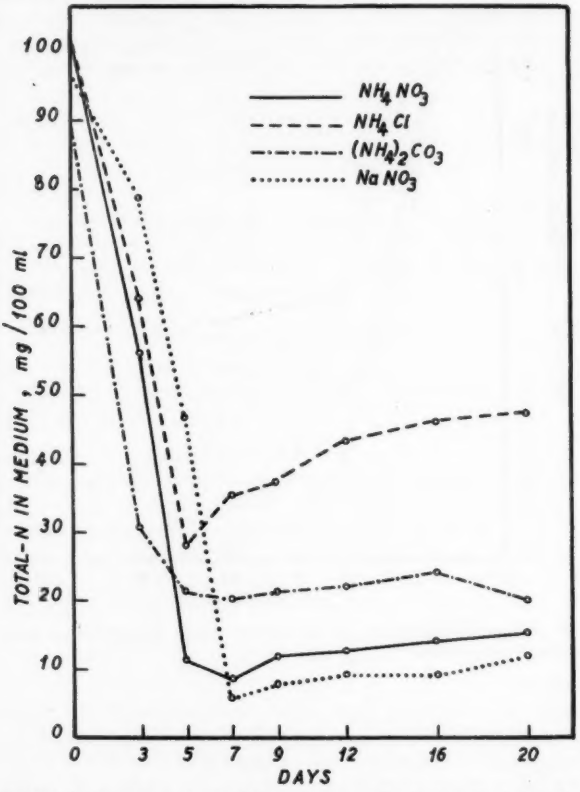


FIG. 2. The total nitrogen content of the culture media during the incubation period.

*Analysis of the Mycelial Felts*

*1. The Protein Content*

As can be noted from Fig. 4, sodium nitrate and ammonium nitrate were more favorable for protein synthesis than ammonium chloride or ammonium carbonate. Highest protein content in the mycelial felts was obtained on sodium nitrate, although protein synthesis started less rapidly than in the other media. Ammonium carbonate was markedly least conducive to protein synthesis. It seems possible to conclude that *Aspergillus nidulans*, under the present experimental conditions, can build up more proteins from nitrate than from ammonium nitrogen (cf. Naguib and Saddik (16)).

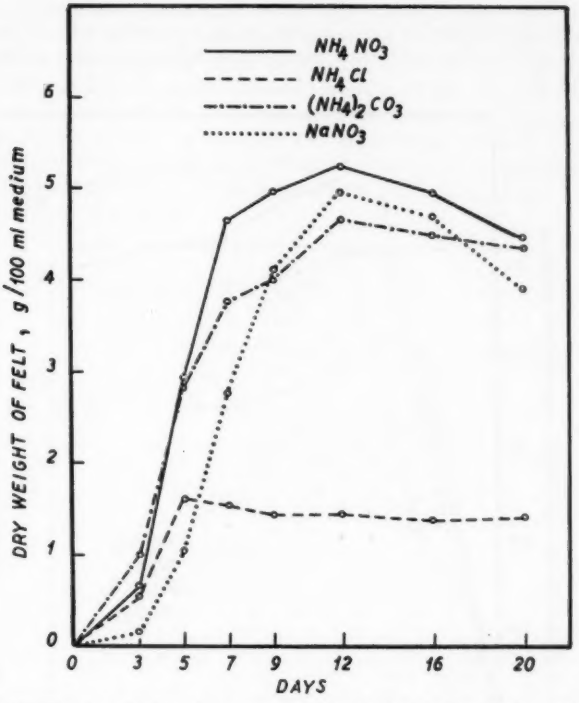


FIG. 3. The dry weights of the mycelial felts during the incubation period.

*2. The Fat Content*

Fat formation, as indicated by Fig. 5, proceeded very slowly at first in all the cultures. Later, it hardly continued on ammonium chloride, but on the other nitrogen sources, the fat content increased steadily. The rate of fat formation was markedly higher in mycelial felts fed with ammonium carbonate or nitrate than in those fed with sodium nitrate, where active fat formation started much later.

## Discussion

It has been claimed that fungi may be classified according to their attitude towards different nitrogen sources (cf. Robbins (19) and Steinberg (22 and 23)). When *Aspergillus nidulans* was grown on sodium nitrate, ammonium chloride, and asparagine, it could absorb and utilize the nitrate and asparagine, but could not utilize ammonium nitrogen (Naguib and Saddik (16)). The pH value in the latter medium fell to 1.7, a value quite suppressive to any further growth, and it was therefore assumed that the inability of the fungus to utilize the ammonium source of nitrogen is due to the large drop in the pH value of the culture media. In the present work, the fungus was

grown on three ammonium salts, namely: chloride, carbonate, and nitrate, and on sodium nitrate for comparison, with a starting pH of about 6. The drift in pH during the incubation period was followed and is plotted graphically in Fig. 6.

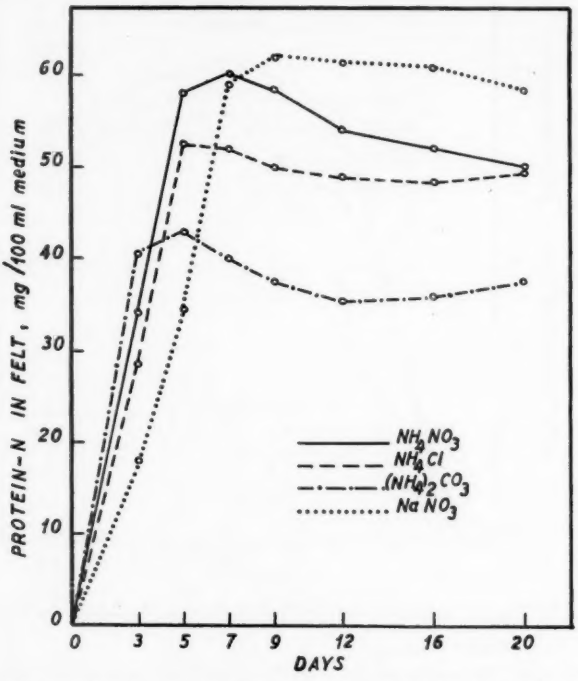


FIG. 4. The protein nitrogen content of the mycelial felts during the incubation period.

The pH value of the ammonium chloride medium dropped quite steadily in the first 5 days until it reached about 2, and then it remained almost constant. Absorption of nitrogen and therefore subsequent protein synthesis proceeded for the first 5 days only. There was no increase in the dry weight beyond this interval, and the fungus continued to absorb sugar very slowly after the fifth day just to maintain a low rate of respiration.

When the fungus was grown on ammonium carbonate, there was an initial drop in the pH value to about 4 during the first 5 days. But this was followed by a gradual recovery almost to the original value. The drop in the pH value was concurrent with the drop in the nitrogen content of the external media, i.e. the removal of ammonium ions. The recovery to the original pH value, on the other hand, may be partly attributed to the possible removal of the carbonate ions through their use as source of carbon. In contrast to growth

on ammonium chloride, the fungus could continue to grow on ammonium carbonate until heavy mycelial felts were produced. Several fungi which were either unable to grow, or only grew poorly, on ammonium salts of strong acids were made to flourish on ammonium sources of nitrogen when the pH of the medium was controlled. This was done either through the use of ammonium salts of weak acids or through the addition of organic acids. Such cases have been reported by Morton and Macmillan (13) with *Scopulariopsis brevicaulis*, Apparoa (1) with *Piricularia oryzae*, and Hacskaylo, Lilly, and Barnett (7) with several fungi.

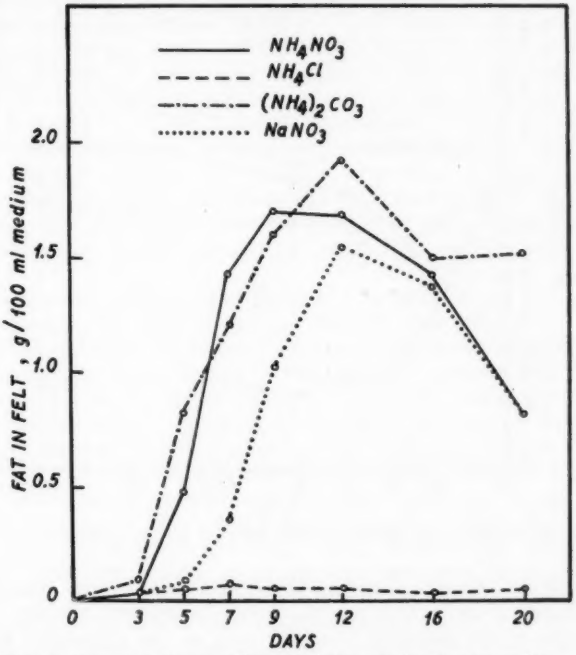


FIG. 5. The fat content of the mycelial felts during the incubation period.

It is interesting here to note that in the ammonium carbonate media, the fungus ceased to absorb nitrogen while an ample supply was still present in the culture media. This occurred in spite of the fact that the pH changed only within reasonable limits. The retarded nitrogen absorption led to the formation of mycelial felts which had strikingly low protein content, compared with those felts formed on other nitrogen sources (Fig. 4). The fact that the fungus could not make use of an ample supply of nitrogen in the culture media after the fifth day of incubation cannot in this case be attributed to a change in pH of the medium. The pH during this period was between 5 and 6. From data given by Singh and Walker (21), it can easily be deduced that

*Aspergillus nidulans* grows well at pH values ranging from 2.8–7.4, in a medium containing ammonium nitrate as nitrogen source. It may be concluded therefore that the present fungus cannot use ammonium nitrogen to the same extent as nitrate nitrogen even if the adverse change in the pH value of the culture medium, which is brought about by preferential absorption of the ammonium ion, is avoided.

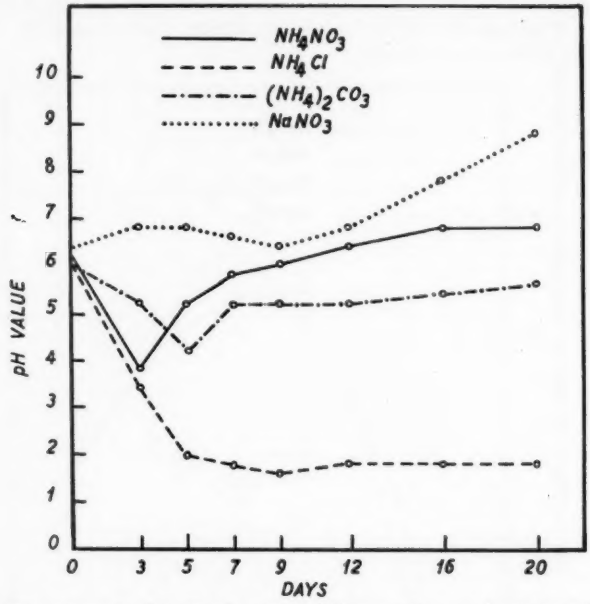


FIG. 6. The change in the pH value of the different culture media during the incubation period.

In spite of the fact that nitrogen absorption ceased in the ammonium carbonate cultures, after the fifth day of incubation, growth indicated by increase in dry weight of the mycelial felts continued at the expense of sugar alone. This led to the formation of heavy mycelial felts with high fat content. It is quite interesting that far better growth could be obtained when ammonium nitrogen was supplied as ammonium carbonate in place of ammonium chloride, but the mycelial felts had a low protein content and a high fat content. In fact, the greatest amount of fat was obtained on ammonium carbonate (Fig. 5). However, increase in weight due to mere accretion of fat cannot be considered as real growth.

When ammonium nitrate was used as nitrogen source, there was an initial drop in pH similar to that observed in ammonium chloride cultures, followed by a rise. Both the drop and the rise occurred while absorption of nitrogen was proceeding, a fact that may be taken as evidence that the ammonium ion was absorbed first and then the nitrate ion (cf. Foster (6) and Morton and Mac-

millan (13)). However, this was further confirmed here when the ammonium and nitrate nitrogen fractions were estimated separately. It was the experience of several workers such as Isaac (11), Haskins and Weston (8), and Pelletier and Keitt (18) that utilization of ammonium nitrate is accompanied by a drop in pH and that this drop can indicate the preferential absorption of the ammonium nitrogen. When the fungus apparently ceased to absorb nitrogen, the medium contained only a small fraction. Sugar uptake also continued until the medium was completely depleted of its sugar content. This was reflected in a rapid growth rate and the formation of a heavy felt compared with ammonium chloride or ammonium carbonate cultures.

The behavior of the fungus on sodium nitrate may reflect some light on what has been observed on ammonium salts in general and ammonium nitrate in particular. No drop in pH similar to that observed in the ammonium media was detected in the sodium nitrate medium, but the pH gradually rose up to nearly 9 during the later period of incubation. Growth started by a lag period, but soon after this phase of poor growth, a steady uptake of both sugar and nitrogen followed. This resulted in a rapid increase in dry weight of the mycelial felts until a maximum near that of ammonium nitrate cultures was reached. The protein content of the fungal mycelia exceeded that of the ammonium nitrate cultures and was maintained over a long incubation period.

It was also observed that absorption of nitrogen did not proceed to completion in any of the cultures. Large amounts were left in the ammonium chloride and ammonium carbonate cultures, while relatively smaller amounts were left in the nitrate cultures. This was followed by increase in the nitrogen content of the culture media through escape of nitrogenous compounds from the mycelial felts. This escape of nitrogen was found to occur while the mycelial dry weight was still increasing in all except the ammonium chloride cultures. It can not therefore be regarded as purely autolytic. This phenomenon has been recorded before with the same organism (Naguib (15)). It has also been reported with other fungi by several workers such as Hockenhull (9), Morton and Broadbent (12), and v. Hofsten (10). It is interesting to add here that the process of nitrogen release from the mycelial cells is affected by the nitrogen source; the greatest amount escaped from felts grown on ammonium chloride, while the smallest amount escaped in sodium nitrate cultures. It may be recalled here that the pH value dropped in the ammonium chloride cultures to a very low value. A correlation may therefore be suggested between this drop in pH and the easy escape of nitrogen from the mycelial cells. It may be of interest to mention in this connection that Mothes (14) has claimed that when nitrate is supplied to fungi or higher plants, nitrogen loss occurs from the former but not from the latter. It may here be pointed out that such loss of nitrogen was not solely correlated with nitrate utilization; it was even more pronounced on ammonium chloride than on nitrate salts.

## Acknowledgment

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# NOTES ON NEARCTIC HEPATICAE

## XVIII. NEW LOPHOZIACEAE FROM THE ARCTIC ARCHIPELAGO OF CANADA[1]

RUDOLF M. SCHUSTER


### Abstract

The difficult genus *Lophozia*, of widespread distribution in the cooler and cold regions of the northern hemisphere, includes a high number of technical species, some of which are very poorly understood. During the summer of 1955 the writer collected extensively on the north coast of Ellesmere Island and was able to study, in the living condition, the high-arctic representatives of this genus. Four species not previously found in the Arctic Archipelago of Canada, three of which are new to the western hemisphere, were collected. Two of these, *L. groenlandica* and *L. opacifolia*, are first reported from the Canadian Archipelago in this paper. Two others, *L. pellucida* and *L. hyperarctica*, appear to be new to science. These belong to the subgenera *Lophozia* and *Massula*, respectively. Since these species are not alluded to in my "Conspectus" of 1951, and since no treatment of them exists in the American literature, the following detailed treatment is provided.


### Introduction

*Lophozia* s. lat. is perhaps the most difficult genus of Hepaticae found in the cooler and cold portions of the northern hemisphere. Unlike *Scapania*, the other large genus of the region, the systematics of *Lophozia* are baffling, and the concept of genus and species remains in some cases very subjective, despite the careful work on both generic classification and systematics of the subgenus *Lophozia* by Buch (5, 6). Schuster (19) has given detailed reasons why *Lophozia* should be delimited broadly, although he was unwilling to go as far as Andrews (1) in merging genera. Recently, Jones (14) has taken an intermediate position, accepting some reductions in rank advocated by the writer (19), such as *Isopaches* to a subgenus of *Lophozia*, but has accepted a number of the segregate "genera", *inter alia*, *Leicolea* and *Barbilophozia*. As regards the distinctness of these groups, accumulated evidence has shown that, on a world-wide basis, these genera are not acceptable, since totally intermediate species continue to be found. For example, see Inoue (11), who has recently clarified the systematics of *Lophozia diversiloba*, a species exhibiting features clearly intermediate between the "genera" *Leiocolea*, *Barbilophozia*, and *Lophozia* s. str., as accepted by Jones (14) and Müller (18). For this reason, and for others inappropriate for documentation in this connection, I continue to use *Lophozia* in the broad sense, as in the standard works by Müller (17) and Macvicar (15).

Controversy regarding species limits is equally continuous; for example, the distinctness of *Lophozia silvicola* Buch, a relatively recently proposed taxon, is challenged by Müller (18), who reduces it to outright synonymy under *L. ventricosa*, and by Jones (14), who would accept it as a mere variety of *L. ventricosa*. Similarly, *L. groenlandica*, a species described by Nees in

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Contribution from the Department of Botany, University of Massachusetts, Amherst, Mass. The research on the present paper was done while the writer held a Guggenheim Fellowship, at Duke University.

Gottsche, Lindenberg, and Nees (10) as long ago as 1844, does not appear as a distinct species except in the very recent works by Arnell (3) and Schuster (Schuster, Steere, and Thomson (25)), yet is apparently fully distinct from both *L. ventricosa* and *L. wenzelii*. Previously *L. wenzelii* had been accepted in a broader sense, including *L. groenlandica*, on the precept of Müller (17). Such shifts in opinion, even by a single worker, suggest that hard and fast opinions as to species limits in this group are not yet advisable. Much more detailed field work will need to be done, as well as culture work, and study of the living "wild" plant. The following study of several critical species continues my earlier work in the group (Schuster (19, 20, 21, 22, 23, 24)). The special value of the present study is that it was based on examination of living plants, in the localities where they were collected. This is the first study of high-arctic Lophoziaceae, *based on living material*. As a consequence, the conclusions here advocated are based on a wider range of characters (including ecological and cytological features) than have been available to previous workers.

It must be admitted, however, that the high-arctic representatives of *Lophozia* remain baffling and their interpretation remains as yet somewhat subjective, owing to a series of interrelated factors that are still not clearly understood, including: (1) evidently sporadic polyploidy, or at least sporadic development of plants with unusually large cells and gemmae; (2) considerably different ranges in oil-body numbers, viv-à-vis "temperate" members of presumably the same species; (3) differences in ecology, associates, and to some extent in pigmentation responses; (4) almost constant sterility, with the consequence that characters derived from the perianths and bracts are rarely available. Particularly puzzling is the fact that considerably different combinations of characteristics occur in the supposedly conspecific members of the same complex from temperate zones. For example, the plants referred to *Lophozia ventricosa* from northern Ellesmere Island (see Schuster, in Schuster, Steere, and Thomson (25)) have fewer oil-bodies, often as few as 5–7 per cell, with the average merely 9–12 in most cases and a maximum, rarely realized, of 14 or 15–16. Yet in "normal" *L. ventricosa* there are supposedly 10–15 or even 11–18 oil-bodies per cell. Furthermore, plants identical with the Alert populations of *L. ventricosa*, collected in the summer of 1960 in Greenland, possessing perianths, have the perianth mouth fringed with teeth and cilia in large part two to three, sporadically four, cells long. Such plants have the perianth mouth of the xylicolous *Lophozia porphyroleuca*, and also show essentially the same (relatively low) number of oil-bodies; see Schuster (21). Yet the pigmentation pattern (no reddish coloration of the stem nor of the postical leaf bases; yellow-brown coloration of the distal parts of the leaves) and the rather orbicular leaves with arched sides eliminate "typical" *L. porphyroleuca* from consideration. The problem arises, thus, of (*a*) how to delimit the arctic phenotypes, vis-à-vis "species" based on phenotypes described from non-arctic populations; (*b*) how broad or narrow a species concept to adopt. I do not think that a stereotyped and simple answer to these problems will be forthcoming. I further doubt that growth experiments of the type carried out on *Lophozia* species by Buch (5) will give any ultimate answer, since such experiments, in the ultimate analysis, only serve

to demonstrate whether two populations differing phenotypically are also genotypically distinct, without proving that the genotypes are sufficiently far removed to represent valid species.

The present study of Ellesmere Island Lophoziaceae is limited to the four species here reported as new to the Canadian Arctic. *Lophozia latifolia* Schuster, also new to the Canadian Arctic, and the other taxa of Lophoziaceae are treated in sufficient detail in the work of Schuster, Steere, and Thomson (25) that any recapitulation of the data there presented is unnecessary here. A complementary study of the *Lophozia* complex as represented in a non-calcareous region, in northwest Greenland, in the Inglefield Bay area, was made by the writer during the summer of 1960; it will be reported on shortly in detail, and that work should be consulted in conjunction with the present paper.

## Descriptions

*1. Lophozia (Massula) hyperarctica* sp. n.

Plantae erectae ad ascendentes, aut repentes, omino virides vel modo lobi supremorum foliorum fusci, crassulissimae et molles. Caulis planatus, crassulus et fortis, pocyicalis facies nigrescens 450–550 (650–800) $\mu$ latus × 350–400 $\mu$ altus; cellulae corticales 2.5–4.5 (5.5) × longiores quam latae. Folia minima ad magnitudinem caulis, non regulariter, tenuiter 2–4 lobata ad 0.15–0.3 longitudinis, latissima (1.5–2.3 latiores quam longa) crassula basi, stabila et rigida; margines integri aut cum 1–2 parvis dentibus, lobi numquam mucronati vel cuspidati. Cellulae 25–32 $\mu$ ad 30–32 $\mu$ in marginibus et in lobis folii; cellulae medianae 35–38 $\mu$ cellulae cum tenuibus vel minime aeque crassulis parietibus; corpora oelifera 6–21 per cellulam, delicatissime segmentatae. Gemmae pallide virides, polygonales, 25–30 × 30–35 (48) $\mu$. Typus: Alert, NE. Ellesmere Island (*Schuster 35142, 35061, 35063a, 35077, 35072a*).

Plants usually scattered, or in small patches, erect or ascending (when growing amidst caespitose mosses) or prostrate with ascending apices (when on bare soil), *pure green or with the lobes of the uppermost leaves golden brown*, rather pellucid, *very fleshy and tender*. Shoots 1.5–1.8 (2.2) mm wide usually, 5–12 mm long, simple or furcate, the subtending leaf hardly modified. *Stem flattened, ± fasciated, elliptical in cross section, stout, soft and fleshy*, whitish green, the postical side blackish pigmented (but *never purplish*) with age, 450–550 (rarely 650–800) $\mu$ wide × 350–450 $\mu$ high, formed of thin-walled cells throughout, 14–16 cells high; dorsal cortical cells narrowly rectangulate, ca. 22–28 to 28–32 (36) $\mu$ wide, *2.5–4.5 (5.5) × as long as wide* (the basal leaf cells 1.5–2 × as wide). Rhizoids frequent, colorless, ca. 18–20 $\mu$ *in diameter*. Leaves obliquely inserted, obliquely spreading, *very small for size of stem* (their length hardly equal to stem width), irregular, usually shallowly two- to three-lobed (for 0.15–0.3 their length) but sometimes shallowly three- to four-lobed (particularly near apices of gemmiparous shoots), *strongly transverse*, from 750 $\mu$ wide × 500 $\mu$ long to ca. 1150–1400 $\mu$ wide × 600–620 $\mu$ long (*from a minimum of 1.5 up to 1.9–2.3 × as wide as long*), rectangulate, *firm and* often rigid, somewhat fleshy at least at base (the basal half two-stratose, with the area *just above juncture with stem often three- to four-stratose*); margins entire, or with one to two low teeth, with prolonged gemmae forma-

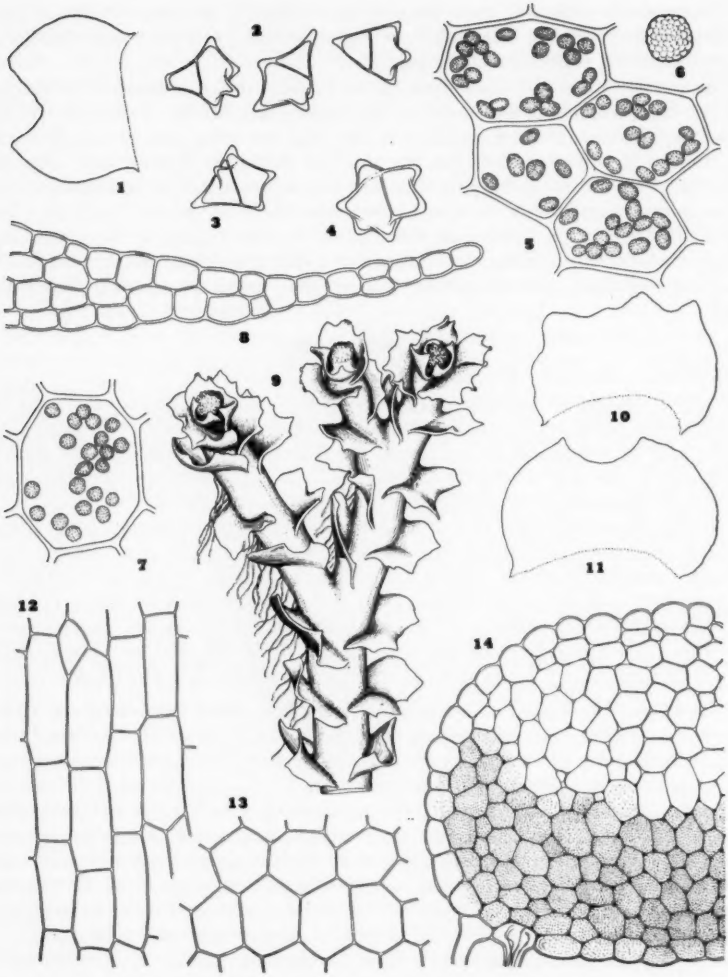


FIG. 1. *Lophozia hyperarctica* Schuster. 1. Leaf (×34). 2–4. Gemmae (×400). 5. Cells from base of leaf lobe, showing oil-bodies (×548). 6. An individual oil-body (×1400). 7. Cell from leaf middle (×570). 8. Longitudinal section of an unusually thick leaf, from base to sinus (from xeromorphic, brownish, compact phase of the species, with rigid leaves) (×150). 9. Lax-leaved, green, gemmiparous, terminally branched shoot (×12.3). 10–11. Leaves (×24). 12. Dorsal cortical stem cells (×220). 13. Basal leaf cells (×220). 14. Cross section of one-half of small, non-fasciated stem, the fuscous-purple medullary cells stippled (×128). (Figures all from type material, from near Alert, N.W. T.; 1–3, 5–7 from *Schuster 35061*; 4, 9–13, from *Schuster 35077*; 8, from *Schuster 35072a*; 14, from *Schuster 35142*.)

tion sometimes bearing scattered teeth; *teeth and lobe apices never sharp and cuspidate;* sinus obtuse to crescentic; lobes rectangular with obtuse or subacute tips to obtuse or rounded at apex. Cells of margins of lobes and of leaf tips from 25–32 (35) $\mu$ to (25) 30–32 $\mu$, almost isodiametric; *median cells* ca. *35–38 (42–44)* $\mu$, almost isodiametric; basal cells 35–38 (44) $\mu \times$ 40–45 (50) $\mu$; cells all very thin-walled or slightly equally thick-walled, virtually *without trigones*, strongly convex; cuticle smooth; oil-bodies usually from *6–18 (rarely 20–21) per cell, finely but distinctly papillose-segmented*, greyish, spherical to ovoid, *from 4 × 5–6 to 5 × 6–8* $\mu$ *or (3.5) 5–7.5* $\mu$, averaging much larger than chloroplasts. Underleaves lacking. Gemmae *pale green*, in masses at apices of uppermost leaves, two-celled when mature, *polygonal to stellate, from 25 × 30 to 28–30 × 33–35* $\mu$, *up to 30 × 48* $\mu$.

Sex organs unknown.

*Type*

Moist clay–shale polygon soil at Alert Weather Station, 82°30′ N. lat., NE. Ellesmere Island, N.W.T. (*Schuster 35142, 35061, 35063a, 35077, 35072a*; the material from these five collections extremely skimpy, coming from the same locality and to be considered the collective type). The material was segregated from admixtures, all collected within a very small radius.

*Ecology*

Occurring around the edges of polygons, in calcareous clay–shale associated with a series of mosses, and the following Hepaticae; *Scapania gymnostomophila, S. cuspiduligera, Solenostoma schiffneri, Cephaloziella arctica, Lophozia muelleri, L. gillmani, L. pellucida, Athalamia hyalina, Sauteria alpina, Aneura pinguis*, and *Preissia quadrata*. Associated are angiosperms such as *Alopecurus alpinus, Eriophorum scheuchzeri* and *E. angustifolium* var. *triste, Juncus biglumis*, etc. The plant occurs in low, poorly drained areas where it is apt to be submerged during the spring snow-melt (in the middle of June), it is found either as isolated, straggling individuals at the bases of *Alopecurus* culms, or straggling among mosses. The plant appears always to occur in isolated stems or small tufts, over silty clay, often in admixture with coarser mosses. It then grows erect and is straggly, with the small leaves very distant on the fleshy, flattened stems. Occasionally a pioneer on damp polygon soil, in full sun, then very short, only 3–8 mm long and compact, with imbricate leaves, the leaves at least toward their apices golden-brown pigmented.

*Variation*

In the Ellesmere Island region around Alert several small collections evidently all referable to this species were made. In the field the plants gave the impression of a small *L. grandiretis*, rather than *L. incisa*, having the same clear green color (but totally lacking all purplish pigmentation of the underside of the stem). In the compact plants (*Schuster 35061*) the fleshy stems were about twice as wide as high (440 $\mu$ high × 800 $\mu$ wide); the cortical cells were thin-walled, ca. 28–32 (36) $\mu$ in diameter dorsally and laterally; the ventral cortical cells were somewhat smaller, 25–30 $\mu$ in diameter, with age blackish- or brownish-walled (and in mature stems the ventral two-fifths of the medulla was also brownish-walled). However, the ventral medullary cells

were scarcely smaller than the dorsal medullary cells (25–36 μ vs. 35–40 μ respectively). The bilobed leaves, on the upper portions of the plants, had the lobe apices pigmented a clear brown! Such pigmentation is quite unknown in *L. incisa*. The cells of the lobes and leaf middle ranged from (25) 30–32 μ up to 35 × 35–42 μ, showed slightly thickened walls, and faintly indicated trigones. The bilobed leaves were small compared to the fleshy stem, ca. 750 μ wide × 550 μ long, entire-margined, much like those of *L. grandiretis*, and two- rarely three-stratose at the base. The cells usually had 15–21 relatively large, finely papillose-granular oil-bodies, ranging from 3.5–5 rarely 7.5 μ and spherical and 4 × 5–6 to 5 × 7–8 μ and ovoid, quite unlike those of either *L. incisa* or *L. grandiretis*. The greenish, polygonal-stellate gemmae ranged from a low of 25–28 × 30–35 μ to 33 × 30 μ up to 30 × 48 μ.

Occasionally, when growing sheltered among caespitose mosses, the plants have the leaves largely unistratose, except at the base, where they are two-stratose. By contrast, the compact sun phases, with pigmented, brownish leaves, have leaves that are much more rigid and which are two-stratose up to above the middle, and three- to four-stratose in the basal fourth or fifth of the leaf; such a leaf is figured in cross section in Fig. 18.

*Differentiation*

Although the distinctness of the species is difficult to establish from such sparse material, the plants are very different from *L. incisa*. The differences in leaf form, in the thickness of the stem, in the oil-bodies, and in the color (pure green in *L. hyperarctica*, in sun phases clear brownish on the lobes of the leaves; pale, bluish green in *L. incisa*, with the sun phases identical in color) strongly suggest that *L. hyperarctica* is a well-marked species. The frequent occurrence over calcareous clay, in the Alert area, suggests that the plant is a calciphyte, whereas *L. incisa* is a marked oxylophyte. *L. hyperarctica* thus not only approaches more closely *L. grandiretis* in coloration, in the extremely stout and flattened stems and in the often distinctly segmented oil-bodies of superior size, but also in its ecology.

The oil-bodies appear to be among the most distinctive features of *L. hyperarctica*. These are finely granular-papillose or segmented, formed of a large number of barely protuberant spherules, exactly as in the subgenus *Lophozia*. Moreover, their size (from a minimum of 3.5–4.5 μ to 5–7.5 μ and spherical or 4 × 5–6 to 5 × 6–8 μ and ovoid), and the number per cell (commonly 6–18, rarely 19–21) closely agree, respectively, with those of the subgenus *Lophozia*. The writer has had occasion to study the oil-bodies in five different collections of this species, and finds that there is no perceptible deviation from these measurements.

Among the species of *Massula* there appears to be some affinity to *L. grandiretis* (in which some phases, at least, show oil-bodies divided into distinct segments) and *L. opacifolia* (and the closely allied *L. incisa*). Although the leaf cells are scarcely larger than those of *L. opacifolia* and *L. incisa*, they differ greatly in content. In the latter two species, numerous, minute, homogeneous oil-bodies occur, giving the cells an opaque, milky appearance (and the whole plant, as a consequence, a peculiar, opaque, greyish- to bluish-green color). When living plants are available, this distinction is easily per-

ceptible. Associated with the numerous, minute oil-bodies is a tendency for *L. opacifolia* and *L. incisa* to blacken when dried. In *L. hyperarctica* (and *L. grandiretis*) no such tendency exists.

*L. hyperarctica*, although similar to small phases of *L. grandiretis*, cannot be identical with this because of the (*a*) smaller leaf cells; (*b*) fewer and different oil-bodies; (*c*) lack of vinaceous pigmentation; (*d*) ability to develop golden-brown pigmentation of at least the lobes of the uppermost leaves. The vinaceous pigmentation, often suffusing all of the ventral half of the stem or more (cortical and medullary cells alike) in *L. grandiretis*, is replaced in *L. hyperarctica* by a black or fuscous pigmentation. The ventral half of the stem in *L. hyperarctica* may appear, at first glance, to be mycorrhizal. However, the dark discoloration appears to be principally (or entirely) due to a cell-wall pigment. The ventral five to six tiers of pigmented medullary cells appear to form a storage system. When these cells are macerated, it is evident that they are densely filled with an opaque, whitish substance (? starch). The ventral cortical cells, and the rhizoids, are slightly mycorrhizal; it is possible that limited mycorrhizal infection also characterizes the lower medullary cells, but no evidence of cellular disintegration associated with mycorrhizas has been found (which is unlike the situation in the subgenus *Lophozia*). The ventral medullary cells are hardly smaller than the dorsal five to six tiers of medullary cells (averaging from 23–26 × 25–35 $\mu$ in diameter and 28–36 (40) $\mu$ in diameter respectively), but the dorsal medullary cells are clear and pellucid, lacking all trace of pigmentation, and clearly lacking any function as a storage system.

I am of the tentative opinion that *L. hyperarctica* and *L. opacifolia* are the high-arctic counterparts of the less strictly northern *L. grandiretis* and *L. incisa*, respectively. It is possible that a similar relationship exists between these pairs of species as between *Cephalozia ambigua* and *C. bicuspidata*. However, the species distinctions between *L. hyperarctica* and *L. grandiretis* are quite strongly marked.

In addition to the Ellesmere collections referred to *L. hyperarctica* above, I would tentatively refer here very sparing plants from northwest Greenland: "Along blocky outcrop of schist, NE. slope of valley, W. of Nuna Lake, Nunatarssuaq," *W. S. Benninghoff 8608*. The few plants seen—almost entirely destroyed in the course of investigation—were found under non-calcareous conditions, judging from the associates (*Marsupella emarginata*, *Gymnomitrium concinnatum*, *Lophozia alpestris*, *Tritomaria quinquedentata*, *Eremonotus minutus*). These plants are few, and since they lack pigmentation and were examined some years after they were collected (thus failed to show the oil-bodies so crucial in separating *L. hyperarctica* from *L. opacifolia*), they cannot be placed with absolute certainty. The following descriptive notes, given *in extenso*, were made from this material.

Plants depressed in growth, *opaque* green, *very fleshy*, devoid of all trace of secondary pigmentation, sparsely branched, freely rhizoidous. Stems *very fleshy*, *strongly flattened*, *narrow-elliptical in section*, pseudothallose in appearance (the leaves with their length averaging less than width of stem), varying from circa 700 $\mu$ wide × 360–420 $\mu$ high, to 900–950 $\mu$ wide × 370–400 $\mu$ high, formed of nearly uniformly thin-walled cells, ca. 15–16 cells high;

cortical cells much smaller than medullary (dorsal cortical 17–20 (23) $\mu$ in diameter × 36–(50)–72 $\mu$ long; ventral cortical 18–20 (25–30) $\mu$ in diameter); medullary cells large dorsally, in five to six layers, ca. (32) 35–48 $\mu$ in diameter, the ventral six to eight layers much smaller, ca. (20) 23–35 $\mu$, *becoming brownish with age* and forming a more or less discrete, ventral, small-celled band. Leaves *irregular*, *coarsely undulate*, mostly two-(three-) lobed, strongly *transversely rectangular*, averaging twice as wide as long (from a minimum of 520 $\mu$ long × 950 $\mu$ wide to 650–700 $\mu$ long × 1300–1400 $\mu$ wide), *similar to those of L. grandiretis in appearance*, the shallow sinus obtuse to crescentic, the lobes subobtuse to subacute, the margins of the lobes with scattered, low, often faint, irregular one- to three-celled, often obtuse teeth, produced largely in association with gemmae-formation, *often virtually edentate*, never sharply spinose-dentate; leaves with distal 0.5–0.7 unistratose, the *basal 0.3–0.4*, *rarely 0.5 bi-*, *locally tri-stratose*, *fleshy*. Cells *small*, *thin-walled*, and virtually *without* trigones, in leaf middle ca. (23) 25–32 × 25–36 $\mu$, mostly rectangular; cuticle thin, smooth. Gemmae in irregular greenish masses at the shoot apices, rather freely produced, one- to two-celled, *very strongly angulate*, the angles drawn out into horns, *often stellate in appearance*, 23–25 × 23–35 $\mu$. Otherwise unknown.

The Greenland plant agrees with *L. opacifolia* in the following critical respects: (1) leaves two- to three-stratose basally; (2) appearance intermediate between *L. incisa* and *L. grandiretis*, in the form and orientation of the leaves, and the very fleshy stem. In these respects it approaches *L. opacifolia*. Indeed, Meylan (16, p. 174) explicitly emphasizes that, "in certain respects, *L. opacifolia* appears more or less intermediate between *L. incisa* and *L. grandiretis*."

In turn, the Greenland plants referred to *L. hyperarctica* differ from descriptions of *L. opacifolia* (as well as *L. incisa*) in the extraordinarily flattened stems, on mature shoots more than twice as broad as high. Note, however, that such fasciation of the stem may occur in the Ellesmere plants of *L. opacifolia*. The leaves of the Greenland variant closely match those of *L. grandiretis* in shape, and in the almost constant bilobing. No spinose teeth are developed (unlike *L. incisa*), but obscure, scattered, marginal teeth occur, especially on leaves that were at one time gemmiparous. I would refer the Greenland plant to *L. hyperarctica* chiefly because no trace of the inflated, sharp, terminal cells of the lobes, which normally characterize the *L. incisa-opacifolia* complex, could be found; because the leaves were, normally, shallowly bilobed, rather than two-to four-lobed; and because the ventral medullary cells of the stem tended to become brownish. However, the intense, fuscous pigmentation diagnostic of the Ellesmere plants is lacking.

It is clear from the preceding that determination of dead, fragmentary materials in this complex will remain baffling and uncertain at times, *even when the same plants, living and with oil-bodies preserved, are easily identified.*

2. *Lophozia opacifolia* Culmann

*Lophozia opacifolia* Culmann, Rev. Bryol. 47:21, 1920; Meylan, Beitr. z. Kryptogamenflora der Schweiz 6 (1): 174, 1924; Müller, Rabenh. Krypt.-Fl. ed. 3, 6 (1): 681, Fig. 212, 1954; Jones, Trans. Brit. Bryol. Soc. 3 (2): 180, 1957; Schuster, *in* Schuster, Steere, and Thomson, Bull. 164, Canadian

Ntl. Museum 30, 1959 (1960).

Similar to *L. incisa*, forming depressed bluish-green patches, or creeping as isolated stems among other Hepaticae. Plants 1.5–2 mm wide, to 1–1.5 cm long, showing occasional furcate branching, *very fleshy, opaque; stems flattened, elliptical in cross section, green* (the postical surface brownish with

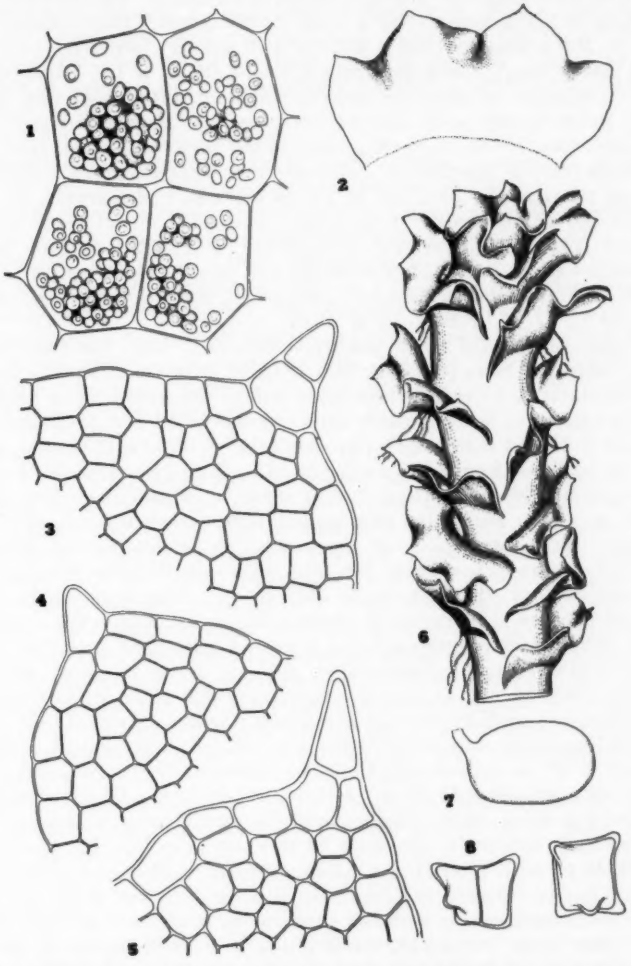


FIG. 2. *Lophozia opacifolia* Culm. 1. Median cells (×735). 2. Leaf (×31). 3–5. Leaf lobe apices, showing weakly cuspidate lobes (×260). 6. Robust, leafy shoot, with strongly elliptical, almost fasciated stem (×19). 7. Stem in cross section (×23.5). 8. Two gemmae (×480). (All figures from *Schuster 35104a*, Mt. Pullen, Ellesmere Island.)

age, due to mycorrhizal invasion of the ventral cortical cells), brittle, ca. 200–230 μ high × 400–450 μ wide; cortical dorsal cells (15) 18–23 (26) μ × (65) 110–150 μ, thin-walled; medullary cells 25–35 μ (rarely to 45 μ) in diameter, pellucid and thin-walled. Leaves barely contiguous to somewhat imbricate, *polymorphous*, very obliquely inserted, two- or partly three-lobed to two- to four-lobed for 1/4–1/3, *strongly transverse* (two-lobed leaves ca. 850 μ wide × 720 μ long to 940 μ wide × 650 μ long; three-lobed, ca. 1050 μ wide × 500 μ long to 1000 μ wide × 600 μ long; four-lobed, ca. 1000 μ wide × 540 μ long), *width averaging 1.2–2.0 (2.5) × the length*, the lobes broadly triangular to acute or sharply angulate, often incurved, *obtuse or pointed*, *entire* (rarely with one to two teeth), *rarely mucronate* at apex (apical cells then 26–28 × 38–46 μ long); *lower portions of leaves two- to three- occasionally four- to five-stratose*. Cells extremely leptodermous, delicate, with minute or no trigones, 23–27 μ, at apices of lobes and margins, 23–28 (30) × 25–35 (40–45) μ medially; occasional one-celled apical teeth formed of cells to 25 × 65 μ, thick-walled; oil-bodies 1.8–2.7 × 2.7–3.6 μ to 2–3 μ, homogeneous and pellucid, 25 or more per cell, usually 30–45 per cell, occasionally even more numerous. Gemmae pale green, one- to two-celled, angulate, 20–25 μ or 15–17 × 15–20 to 18 × 26 μ.

Dioecious. Antheridial leaves weakly ventricose at base, the undivided base suberect, only the lobes patulous; two to three or more antheridia per bract; stalk uniseriate(?). Female bracts often widely separated from each other, similar to vegetative leaves, mostly three- to four-lobed, somewhat asymmetrically so for 0.25–0.4 their length, somewhat fluted, often with sinuses narrowly reflexed or gibbous, from 1385 μ wide × 875 μ long (when bilobed) to 1450–1550 μ wide × 890–1000 μ long (when three- to four-lobed); their margins edentate or with a few rather thin-walled, differentiated, large-celled, sharp, unicellular teeth; lobe apices at times with well-differentiated, large-celled teeth. Perianth mouth slightly lobulate, subentire to remotely denticulate with *one-celled teeth*, the *teeth mostly only 20–60 μ long*, occasionally 60–80 μ long or even larger (13). Perianth mouth cells occasionally even more differentiated (Greenland plants) and the terminal cells then free for over 0.5 or even all their length; thick-walled, spinous, from 18–21 × 85–92 μ to 23 × 100–140 μ (4–6× as long as wide). Seta 300–330 μ in diameter, 8–10 cells in diameter, with 35–36 rows of epidermal cells (each ca. 24–28 μ in diameter) and gradually somewhat larger interior cells (the central appreciably larger and 42–50 μ in diameter). Capsules *perfectly spherical*, ca. 700 μ in diameter. Wall tristratose, 45–48 μ thick (inner two layers 10–12 μ thick each; outer 23–25 μ thick, thus fully as thick as two interior layers combined). Epidermal cells essentially quadrate to short-rectangulate, ca. 25–28 to 23–25 × 30–38 μ, with moderately strong nodular thickenings, two to three (four) per longer (usually longitudinal) wall, zero to one (two) per shorter (usually transverse) wall; nodular thickenings usually extended as short spurs on the outer (free) tangential walls. Inner cell layers of elongated, less regular cells, where rectangulate 12–15 μ broad usually, with numerous rather narrow, complete semiannular bands. Elaters 7.2–8.5 (9.0) μ in diameter × 100–135 μ long, loosely bispiral. Spores large, 14.5–18 μ up to 18–20 μ in diameter.

*Type*

Alps, Canton Bern, Switzerland (*Susten*).

*Distribution*

A restricted known range in the Alps of central Europe (7, 8, 16) from Savoy, the Tirol (Darmstädter Hut), to Switzerland (several stations; type), and possibly in Austria (Steiermark), according to Müller (18). Arnell (2) reports it common in the Tornetrask area of Sweden and (3, p. 126) states it is "probably common in the fjeld area" of Norway. Jones (13, p. 180) reports it as common in the Cairngorms, Aviemore, Scotland, and from Coire and t'Sneachda, Scotland.

In North America known from:

ELLESMERE ISLAND: North slope of Mt. Pullen, 5 miles S. of Alert, ca. 82°25′ N. lat., NE. Ellesmere Island (*Schuster 35104a*).

NORTHWEST GREENLAND: Kânâk, Red Cliffs Peninsula, Inglefield Bay (*Schuster 45603a*); Siorapaluk, Robertson Bugt (*Schuster 45720*).

LABRADOR: "L'anse au Clair" (*Waghorne 3, Sept. 7, 1894*!); a very few plants, admixed with *Eremonotus minutus* (the latter determined by Underwood as *Diplophyllum dicksoni*). The Labrador plants were evidently collected from the face of a damp-to-dry rock, occurring admixed with *Eremonotus minutus, Mylia taylori, Cephalozia leucantha, Blepharostoma trichophyllum.* These plants disagree in some respects with the type (for the discrepancies see discussion under Differentiation).

*Ecology*

Supposedly restricted to montane areas, from altitudes of 1700 m upwards, "on earth with a prolonged period of snow-cover" but in Scotland found at elevations of above 950 m, also in areas with prolonged snow-cover.

In northern Ellesmere, where the species occurs within 525 miles of the North Pole, I have found the species restricted to moss-tundra, where it occurs among mosses, associated on calcareous clay–shale slopes fed by permanent snowbanks, in areas where the snow persists until after the middle of June; here it is associated with *Lophozia quadriloba, L. heterocolpa, Anthelia juratzkana, Blepharostoma trichophyllum brevirete, Odontoschisma macounii, Tritomaria heterophylla*, etc. The plant here occurs on ground where the permafrost is never more than 4–8 in. below the surface, and where air temperatures during the snow-free season (50–60 days or less) range usually between 28–38° F.

Unlike the closely allied *L. incisa* the species evidently is tolerant of calcareous conditions. However, the Greenland collections all came from very peaty, acid sites.

*Differentiation*

Culmann (7) apparently was hesitant about the status of *L. opacifolia*, since he described it as follows: "Subspecies aut varietas nova Lophoziae incisae." It was described as differing from the xylicolous "typical" *L. incisa* in having (*a*) the leaves with the base, up to the middle, two- to five-stratose, thus quite opaque and fleshy; (*b*) perianth-dentate, not ciliate, at mouth; (*c*) spores larger, 18–19 $\mu$ in diameter. *L. opacifolia* has been variously con-

sidered to be either a distinct species or a variety of *L. incisa*. The latter disposition would be warranted, if it can be shown that the spore size is not constant, since *L. incisa* occasionally has leaves somewhat fleshy at base. This is admitted by Culmann (8, p. 40), who states that in the lignicolous "typical" *L. incisa* he has seen plants with the majority of leaves unistratose to the base, but with "une feuille présentant quatre assizes des cellules vers la base." From this it can be seen that the claims for recognition of *L. opacifolia* are not very strong.

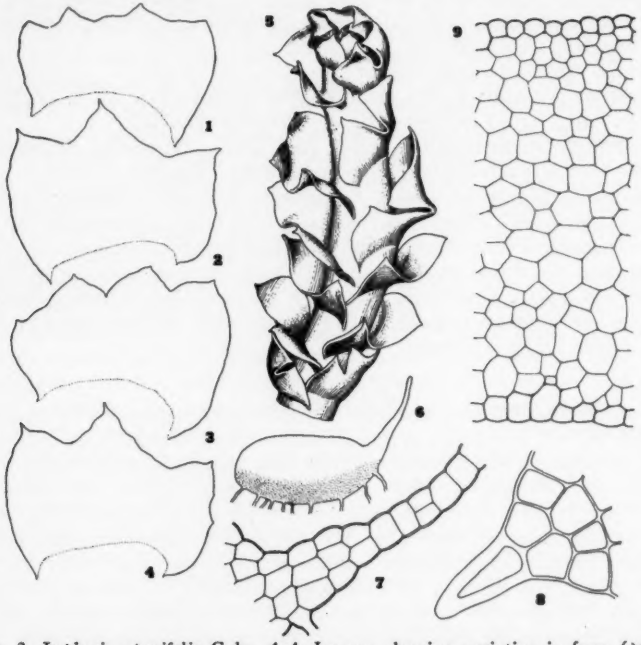


FIG. 3. *Lophozia opacifolia* Culm. 1–4. Leaves, showing variation in form (×24.5). 5. Sterile shoot (×18). 6. Stem cross section, the stippled area more or less mycorrhizal (×41). 7. Base of leaf seen in cross section in 6 (×133). 8. Mucronate apex of leaf lobe (×260). 9. Section through stem middle (×143). (Figs. 1–4, 9, from *Schuster 35104a*, Mt. Pullen, Ellesmere Island; 5–8, from *Waghorne No. 3*, 1894, from Labrador, transitional to normal *L. incisa*.)

*L. opacifolia* can be confused only with *L. incisa*, of which it appears to be an arctic-alpine and high-arctic derivative. The plant has the facies of an opaque, non-spinose form of *L. incisa*, but differs in several significant features, chief among them: (*a*) the leaves "tend" to be three- to five-stratose towards the base and much broader than long, frequently attaining a width of twice the length; (*b*) the triangular and often broad leaf lobes are edentate, with the lobes acute or subacute to weakly mucronate at the apex, the apical cell frequently thick-walled and differentiated from the other cells, but never

long and spinous; (*c*) the perianth mouth is subentire, with scattered one-celled teeth 20–60, occasionally 60–80 $\mu$ long, or even larger, but never with the narrow cilia typical of *L. incisa*; (*d*) the spores are larger, ranging from 14.5–18 $\mu$ to 18–19 up to 20 $\mu$ (vs. 12–15 $\mu$ in *L. incisa*). Admittedly occasional forms appear to be transitional between the two species.

As I have pointed out (25), the cells of *L. opacifolia* agree with those of *L. incisa* in having numerous, minute, homogeneous oil-bodies. The Ellesmere plant had leaves which were often three- to four-lobed and twice as wide as long, or even 2.5 times as wide as long, and had lobes that were edentate, although often mucronate at the apex. However, the leaves of the Ellesmere plant are only two- locally three-layered at the base, while *L. opacifolia* supposedly has the leaves three- to five-stratose towards the base. I do not think this criterion is a critical and constant one; in any eventuality, the abundant Greenland collections I have made of *L. opacifolia* fail to show three- to five-stratose leaf bases, even though they have somewhat larger spores than in *L. incisa*. In fact, most of the characters of *L. opacifolia* are subject to a good deal of variation, and I wonder if it might not prove wiser to regard it merely as an arctic subspecies of *L. incisa*. Against such a conclusion is arraigned a mass of data derived from the perianth mouth and from the sporophyte. The above diagnosis of the perianth mouth is derived partly from Müller (18) and Jones (13), and partly from the Greenland plants, in which the one-celled cilia of the perianth mouth may be extraordinarily differentiated and formed of cells, prominently thick-walled distally, as much as 23 × 100–140 $\mu$ long; such cilia agree with those of *L. incisa*; see, e.g., Müller (18, Fig. 211*f*). Jones (13) shows that in the Scottish plants of *L. opacifolia* the marginal cells of the perianth are 60–80 $\mu$ long, while Müller states they are only 20–60 $\mu$ long in the material from Switzerland. Again the differences between the two "species" here appear to be purely quantitative and subject to intergradation. The Greenland plants also appear to show consistently spherical capsules, while the sporangium in *L. incisa* is described as "ovoid" (9). The spores of *L. incisa* are supposedly 12–15 $\mu$, those of *L. opacifolia* 18–19 or 18–20 $\mu$ in diameter. However, the Greenland plants have them 14.5–18 $\mu$ in diameter! The Greenland collections thus are particularly critical, since they combine, in various ways, the characters of both *L. incisa* and *L. opacifolia*. They do not show the high incidence of extremely broad, three- to four-lobed leaves of the Alert collection of *L. opacifolia* (which is shown in Figs. 2 and 3). These plants will again be discussed in another connection, so perhaps can be put aside for the moment.

The material from Labrador (here assigned to *opacifolia*) is also somewhat of a problem. It has the exceedingly broad leaves (width 1.2–2.0 times the length) of *L. opacifolia*, and the leaves vary from simply two- to four-lobed in most cases, with broad, ovate-to-triangular, entire lobes. However, in a few cases, at least, suggestions of one-celled lateral teeth are found, and on occasional leaves the lobes terminate in a one- to two-celled, sharp, apiculum (typical of *L. incisa*). The Labrador plants also have most leaves only two-stratose at base, some three-stratose for a short distance (a condition thus intermediate between "normal" for *L. incisa* and "normal" for *L. opacifolia*).

In the final analysis, the chief reason for assigning this material to *L. opacifolia* lies in the exceedingly stout, very fleshy, and strongly flattened stem, whose width averages at least one-third the width of the shoot. It is perhaps not a coincidence that these plants should combine some of the characters of both species, and occur in the transition region north of which *L. incisa* "drops out" completely.

Study of material from decayed wood (Labrador: Forteau, *Waghorne 6*) representing *L. incisa* shows only minor differences from the plants referred to *L. opacifolia* above. The leaves "tend" to be considerably more spinous-dentate (but many leaves are simply two-lobed, with the lobes subacute, the leaf width greater than the length); the stem "tends" to be slightly less flattened and less fleshy; the leaves are at most bistratose for a short distance at base. Study of a series of plants thus shows that, at and approaching the Tundra, *L. incisa* "tends" to go over into *L. opacifolia*. This suggests the latter is a mere ecotype or variant of high altitudes and (or) high latitudes.

*3. Lophozia (Lophozia) pellucida* sp. n.

*Lophozia pellucida* Schuster, *in* Schuster, Steere, and Thomson, Natl. Museum Canada (1959), Bull. 164, 34, 1960; *nomen nudum.*

Plantae repentes, or humiliter crescentes, pellucidae, pallide virides ad fuscas, 1750–2200 $\mu$ latae. Corticales cellulae caulium plerumque 16–20 $\mu$ latae × 90–175 $\mu$ longae, pellucidae. Folia remota ad contigua, quadrato-rotundata, 1.1–1.7 latiora quam longa, bilobata, lobi saepe rotundi vel obtusi. Cellulae magnae, parvis cum trigonis, marginales 35–40 $\mu$, medianae 35–42 × 40–50 $\mu$; corpora oelifera per cellulam anguste segmentata. Gemmae similes colore lobis folii, virides ad fuscas magnae. Typus: Alert, NE. Ellesmere Island (*Schuster 35141*).

Plants decumbent to prostrate, with crowding ascending to suberect, slender, scattered or in loose patches, *pellucid, light green or* (with age) *clear chestnut brown, the postical leaf bases sometimes lightly vinaceous.* Shoots slender and small, 1750–2000 (2200) $\mu$ wide × 1–2.5 cm long, sparingly and very diffusely branched. Stems *slender*, almost terete to very slightly flattened, 245–325 (425–500) $\mu$ in diameter, *10–12* (13–14) *cells high*, flexuous and sinuous, *rather soft* and somewhat translucent, pale green to (with age) brownish; *ventral cortical cells linear-rectangulate*, (55) 90–175 $\mu$ long × 16–20 (23) $\mu$ wide (ca. 4–8:1); dorsal cortical cells *elongate* (48) *65–110* (120–160) $\mu$ *long* × (18) 21–26 (26–28) $\mu$ wide, rectangulate, ca. 3–6× as long as wide, *scarcely thick-walled, pellucid;* dorsal medullary cells pellucid, 23–26 $\mu$ high, in five to six tiers; ventral medullary cells notably smaller, usually 13–19 $\mu$ in diameter, in three to five tiers, partly destroyed by mycorrhizas, distinctly smaller than ventral medullary cells. Rhizoids scattered and not abundant, colorless to pale brownish, their bases rarely a slight vinous red, *17–23 $\mu$ in diameter. Leaves distant to contiguous, very obliquely inserted, and quite evidently decurrent antically*, broadly orbicular-ovate to quadrate-rotundate, from ca. 850 $\mu$ wide × 750 $\mu$ long to 1050–1150 $\mu$ wide × 800–950 (1100) $\mu$ long, to 1200–1220 $\mu$ wide × 700–850 $\mu$ long (width 1.4–1.7× the length) up to 1300–1350 $\mu$ wide ×950–1000 $\mu$ long (*width ca. 1.1–1.35× the length*), *nearly flat* or slightly concave to saucer-shaped, but not canaliculate, *usually bilobed* (a very few

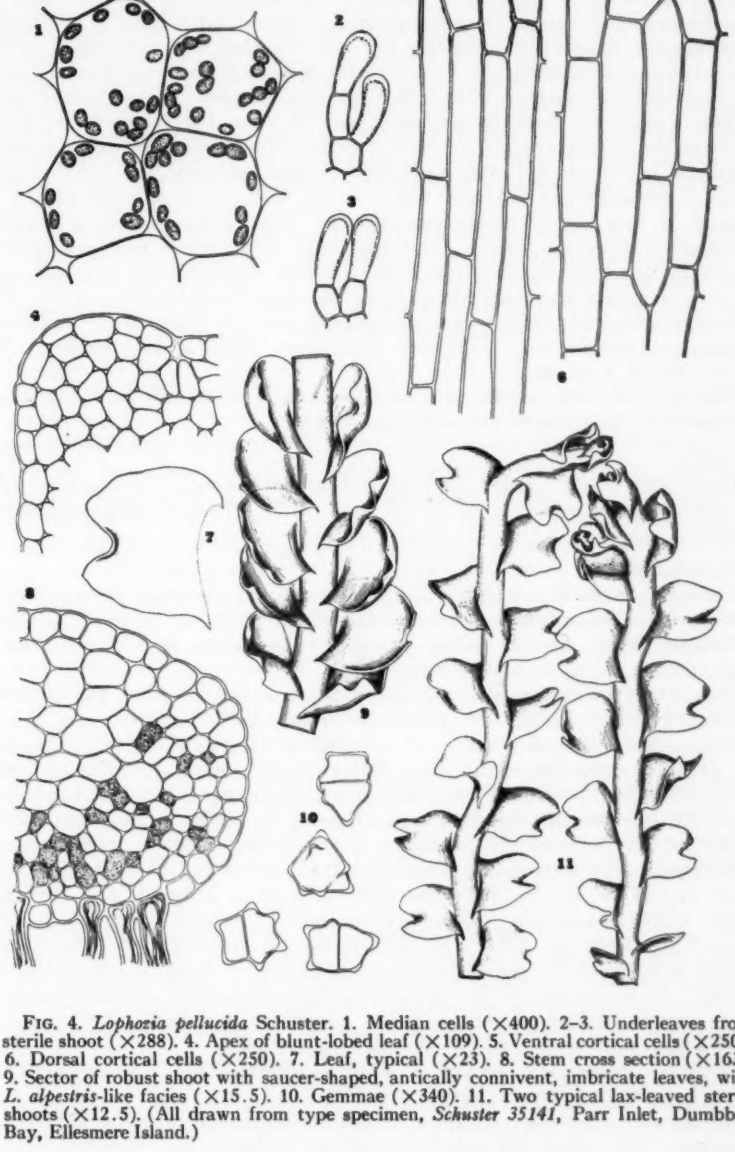

FIG. 4. *Lophozia pellucida* Schuster. 1. Median cells (×400). 2–3. Underleaves from sterile shoot (×288). 4. Apex of blunt-lobed leaf (×109). 5. Ventral cortical cells (×250). 6. Dorsal cortical cells (×250). 7. Leaf, typical (×23). 8. Stem cross section (×163). 9. Sector of robust shoot with saucer-shaped, antically connivent, imbricate leaves, with *L. alpestris*-like facies (×15.5). 10. Gemmae (×340). 11. Two typical lax-leaved sterile shoots (×12.5). (All drawn from type specimen, *Schuster 35141*, Parr Inlet, Dumbbell Bay, Ellesmere Island.)

trilobed), entire-margined or faintly crenulate locally due to projecting cells; sinus descending (1/8–1/6) 1/5–1/4 (1/3) the leaf length, *obtuse to rounded*, sometimes mostly crescentic, *often somewhat reflexed;* lobes broadly triangular, *often obtuse or blunt to (occasionally) distinctly rounded*, with occasional to frequent ones acute to subacute. Cells *pellucid, with walls becoming distinctly clear chestnut brown with age, extremely thin, the trigones small to moderate but never bulging*; cells very large, *the marginal ones 35–40* $\mu$, the subapical 35–40 $\times$ 35–50 $\mu$, the *median (33) 35–42 (45) $\times$ 40–50 (55)* $\mu$; *cuticle smooth*; oil-bodies usually (7) 9–18 per cell, *very small for cell size*, spherical and 4.5–5.5 $\mu$ to ovoid or ellipsoid and 4.5 $\times$ 7–9 $\mu$ or 5–5.5 $\times$ 6.5–7.5 $\mu$, a few to 5.5 $\times$ 8 or 5 $\times$ 9 $\mu$; finely papillose-segmented in appearance, nearly smooth; chloroplasts few and smaller than oil-bodies. *Underleaves frequent, minute*, of one to two stalked, slime papillae. Gemmae rather scarce, in small, *pale green to very pale brownish* masses from the uppermost leaves, one- to two-celled, *polygonal to stellate*, rather thin-walled and with only weakly thickened angular protrusions, variable in size, *large*, from 25–26 $\times$ 28 $\mu$ to 25 $\times$ 32–35 $\mu$ to 34–35 $\times$ 36–40 $\mu$.

Dioecious. Female bracts two- to three-lobed, similar to leaves; a small bracteole usually present. Otherwise unknown.

*Type*

South end of Parr Inlet, Dumbbell Bay, on snow-fed, calcareous, solifluction slope, near Alert (82°30′ N. lat.), NE. Ellesmere Island (*Schuster 35141*).

*Distribution*

Known from the type locality, and from a series of paratypes from the same general region in northern Ellesmere Island; at all times confined to damp calcareous slopes, usually below "hanging," persistent snowbanks. Also a single known station in northern Sweden.

ELLESMERE ISLAND: Clay–shale slope, below snowbank, Mt. Pullen, 800–900 ft, 82°25′ N., 62°20′ W. (*Schuster 35786, 35312, 35786a*); clay–shale calcareous flat, outwash plain N. of Mt. Pullen, 82°26′ N., 62°15′ W. (*Schuster 35382a*); steep clay–shale slope, S. end of Hilgard Bay, 82°26′ N., 63°25′ W. (*Schuster 35931, 35934a*); calcareous clay slope below hanging snowbank, 2.5 miles W. of Cape Belknap, adjoining mouth of Colan Bay, 82°32′ N., 62°30′ W. (*Schuster 35205a, 35213, 35205, 35207, 35208*); calcareous clay–shale slope, valley between Mt. Olga ("Peak 1") and Mt. Erica ("Peak 2"), ca. 82°22′ N., 64°15′ W., ca. 5–6 miles SW. of head of Hilgard Bay (*Schuster 35549, 35520*); damp clay–shale slope adjacent to polar ice cap, 1.5–2 miles W. of Cape Belknap, 82°32′ N., 62°20′ W. (*Schuster 35634b, 35626*, and *35638a, p.p.*, among *L. pellucida minor*); head of Parr Inlet, S. end of Dumbbell Bay, 82°30′ N., 62°20′ W. (*Schuster 35141c, p.p.*, with *Arnellia fennica*); on calcareous solifluction slope, below persistent snowbank, NW. side of "The Dean," 82°25′ N., 62°10′ W. (*Schuster 35167*).

SWEDEN: Jukkasjärvi socken, Abisko National Park, Karsavaggejokk, Torne Lappmark (*Persson, July 29, 1944*!).

The Swedish plants are not quite typical, although they cannot be referred elsewhere. They show the pale brownish to golden-brown pigmentation of

the upper portions of the leaves; have generally quite large cells (although the basal portions of the leaves, and many leaves on some plants, may possess cells averaging less than 35–38 $\mu$ wide); are nearly leptodermous; have broad and transversely suborbicular-ovate leaves, which are clearly wider than long; have shallow sinuses that may be slightly reflexed; have the thin-walled, elongated cortical cells of the species and the almost colorless gemmae that become light brownish with age. The gemmae show a great variation in size, and may be as little as 25–27 $\times$ 29–32 $\mu$ (perhaps somewhat immature), but the largest are up to 30–35 $\times$ 35–46 $\mu$; they tend to be more sharply angulate than in the type material.

It deserves emphasis that at least in the North American Arctic this species is chiefly one of recently emerged, highly calcareous lowlands, giving the impression of recently having evolved by polyploidy. In the summer of 1960 I searched repeatedly for it in northwest Greenland (77–78° N. lat.), in a non-calcareous area, without being able to find any trace of it.

*Ecology*

Apparently uniformly calciphilous, occurring under a very limited variety of conditions, either on moss-covered, damp, calcareous clay–shale slopes, above which no persistent snowbanks exist, or (more commonly) on seepage-moistened, calcareous clay–shale slopes, situated below hanging, persistent snowbanks or firn-ice-filled valleys. The plants rarely form pure patches, but occur almost constantly among or with the conspicuous reddish-brown or cinnamon-colored moss *Holmgrenia chrysea*, sometimes with *Equisetum variegatum* (at its northernmost known limit at 82°32′ N. lat.), and with a variety of Hepaticae, chief among them: *Arnellia fennica*, *Blepharostoma trichophyllum brevirete*, *Cephaloziella arctica*, *Aneura pinguis*, *Lophozia gillmani*, *L. muelleri* and *L. badensis*, *Athalamia hyalina* (rarely *Sauteria alpina*), *Preissia quadrata*. These preceding species occur admixed in very damp sites, where the *L. pellucida* occurs under hygric conditions. On steeper, better-drained slopes, some of the preceding species may be associated, as well as *Lophozia alpestris major*, *L. quadriloba*, *Scapania gymnostomophila*, *Solenostoma polaris*, *Cryptocolea imbricata*, rarely *L. heterocolpa harpanthoides*, and *Moerkia flotowiana*. Associated angiosperms are usually *Papaver radicatum*, *Salix arctica*, *Saxifraga caespitosa* and *oppositifolia*, more rarely *S. flagellaris*. Associated mosses are, *inter alia*, *Drepanocladus revolvens*, *Hypnum bambergeri*, *Distichium capillaceum*, *Cinclidium arcticum*, *Didymodon recurvirostris*, etc. (25).

*Differentiation*

*L. pellucida* differs at once from the other species of the subgenus *Lophozia* in the extremely large and pellucid cells and in the broad, occasionally to often blunt to rounded, leaf lobes. The plant has the strongly oblique, pellucid, broad leaves characteristic of the subgenus *Massula*; it also has leptodermous to slightly thickened, strongly elongated, narrow cortical cells, much as in the majority of species of *Massula*. The very narrow, ventral cortical cells (4–8, rarely to 10 times as long as wide) are virtually without a parallel in the subgenus *Lophozia*; they occur in lax as well as in rather dense-leaved phases. The species typically also has the flexuous, soft, straggling and sinuous

stems of such species of *Massula* as *L. marchica*. Yet the anatomy of the medulla is clearly that of the subgenus *Lophozia*, and the oil-bodies are as in this in size and form, as well as in number. With age, and in sun, the plants acquire a clear brownish pigmentation, occasionally supplemented by a slight vinaceous tinge of the postical leaf bases, unlike our other species of the subgenus *Lophozia*; furthermore, the rhizoids are broad in diameter and unusually sparse. The leaves are also distinctive in being usually nearly plane, but with the rounded sinus often narrowly reflexed.

From the foregoing it is clear that *L. pellucida* occupies an isolated position in the subgenus *Lophozia*, showing some of the characteristics of *Massula*. It cannot readily be derived as a putatively polyploid race from any other species of these subgenera. Although not known with mature reproductive organs, it is a well-marked and readily recognizable taxon in a group where obviously distinctive taxa are very few. If, on the basis of the very large leaf cells, it is sought in *Massula*, it may be sought under *L. obtusa* (because of the often obtuse or rounded leaf lobes and somewhat reflexed sinus). It differs at once from this species in the form and number of the oil-bodies, in the much larger leaf cells, the narrower and more elongated cortical cells of the stem, and in the stem anatomy.

On the basis of the bilobed leaves with frequently to commonly blunt or rounded lobes, weak shoots of *L. pellucida* are subject to possible confusion with *Gymnocolea*. The leaf cells, however, are much larger than in the latter, and possess thin walls and distinct trigones.

This resemblance to *Lophozia* (*Massula*) *obtusa* and the *Gymnocolea inflata*, based on the leaf shape, is further enhanced by the fact that minute and inconspicuous underleaves regularly occur in *L. pellucida*—another anomalous feature for a species of the subgenus *Lophozia*. These are inserted on normally wide ventral merophytes (usually two to three, rarely four, cells broad), and consist either of a single clavate slime papilla on a one-celled stalk, or of two such papillae, each on a stalk cell. In the latter case, the stalk cells are united for a short distance at base. Occasionally a single stalk cell may bear two slime papillae inserted on it (one sometimes in turn on a one-celled stalk). Similar underleaves commonly occur in *Gymnocolea*, while considerably larger and more elaborated ones are frequent in *L.* (*Massula*) *obtusa*.

Among the species of the subgenus *Lophozia*, a distant relationship with *L. alpestris* and *wenzelii* is possible. Although the majority of less robust shoots of *L. pellucida* have obtuse and blunt to rounded leaf lobes, separated by a rounded sinus, large, mature shoots bear at least some (and often many) leaves in which the sinus is exceedingly shallow and crescentic, descending at most to 1/6 the leaf length. Leaves of this type may also be somewhat antically secund and slightly to distinctly concave, with somewhat incurved leaf lobes (Fig. 4.9). Such *L. alpestris*-like leaves occur sporadically and sometimes rather frequently; they may be very broad (1200–1220 $\mu$ wide $\times$ 700–850 $\mu$ long, or even 1500$\times$1050 to 1550$\times$900 $\mu$), thus ca. 1.4–1.7 $\times$ as wide as long, particularly on robust shoots with stout stems. Associated with the ability to develop such leaves is the usual development of a concave, weakly saucer-shaped leaf form, and the general presence of a warm, chestnut-

brown coloration, recalling that of *L. alpestris*. It is hardly possible, however, that *L. pellucida* can be derived from *L. alpestris*.[2]

The gemmae of *L. pellucida* are usually produced rather sparingly. They are usually greenish, even on lightly brown-pigmented plants. In the case of deeply chestnut-brown plants, the gemmae may be slightly brownish pigmented, but never the bright reddish-brown or carmine characteristic of those of *L. alpestris*. This is the only species of the subgenus *Lophozia* in which the pattern and nature of pigment production of the gemmae correspond with those characterizing normal leaf cells, although a higher light intensity in the gemmae than in the leaf cells appears necessary to initiate pigment production. At least, it is common to find distinctly pigmented plants in which the gemmae have nearly colorless walls. Even when pigmentation of the gemmae develops, it usually develops tardily.

*L. pellucida* is definitely dioecious. The few female plants seen had produced archegonia, which evidently were not fertilized, since neither bracts nor bracteole had fully matured, nor had any trace of a perianth developed. Such unfertilized gynoecia in all cases were subtended by one to two innovations. The more robust stems occasionally undergo almost perfectly dichotomous forking. In such cases, it is difficult to distinguish the branch from the continuation of the main axis, since the leaf subtending the branch is neither narrowed nor otherwise modified.

Although the extraordinarily large size of the cells is very diagnostic of "typical" *L. pellucida*, deviations from the norm are frequent, and may be partially the result of differences in environmental conditions. The plants from wet sites generally are lax-leaved, and have cells 35–40 occasionally 35–45 $\mu$ in the lobes. However, occasional forms from steep, well-drained slopes (*inter alia*, *Schuster 35931*, *35934a*) may be equally robust, although relatively fleshy, with dense leaves; in such the apical and subapical cells may be 35–40, occasionally even 45 $\mu$, or (in other cases) average 35–42 $\mu$.

However, on clay–shale slopes that are merely damp, *L. pellucida* may occur as a smaller, more compact phase, with the apical cells 33–45 $\mu$, growing admixed with plants which are very similar, but have smaller cells (23–28, occasionally 30 $\mu$ in leaf apices, in, *inter alia*, *Schuster 35634c*, *35638a*, *35638*). A few plants from similar sites, however, are quite transitional in cell size; for instance, in plants of *Schuster 35820* the cells may be 28–36 $\mu$ in diameter in the leaf apices, thus approaching "typical" *L. pellucida*, or even (*Schuster 35823*) up to 30–38 $\mu$ in the leaf tips. If it were not for the cell size of these latter plants (locally admixed with "normal" large-celled *L. pellucida*) one would at first appear to be dealing with two distinct species. In fact, the smaller-celled plants bear a close similarity (in the green, nearly leptodermous phases)

[2]The lack of a close relationship to *L. alpestris* is also evident from study of the material of *Schuster 35167*. The sparse plants occur here with admixed plants of *Lophozia alpestris major* (in which the leaves are virtually devoid of pigment, the leaf lobes and sinuses angular and usually sharp, the gemmae reddish, and the marginal cells of the leaf tips ca. 23 $\mu$). The plants of *L. pellucida* show the warm reddish-brown pigmentation of the leaves to a high degree, yet the gemmae are a very pale brownish; the marginal cells of the leaf tips average 34–38 $\mu$. The two taxa are obviously and clearly distinct when plants from the same tuft, growing under identical conditions, are compared. If a relationship to the *L. alpestris-wenzelii* complex occurs at all, it may be rather with *L. wenzelii*.

to *L. ventricosa*, and were at first tentatively referred to a "var. *grandiretis*" of this species. A separation between these smaller-celled plants, referred subsequently to *L. pellucida* var. *minor*, and typical *L. pellucida*, is, however, not always easy to effect. This is especially the case when gemmiparous leaf lobes of the "var. *minor*" are studied, in which the cells of the lobes are often considerably enlarged. The fact that an extremely close affinity occurs is well established by the identity in facies of the two plants: both produce a warm brownish pigmentation of the leaf lobes, at times extending from the leaf lobes almost to the leaf bases (but becoming less intense basally); both have gemmae that are greenish when immature, and which may remain yellowish green in shade extremes, but almost always develop a warm brown pigmentation (identical with that of the leaf lobes). *The similarity in reaction of the cells of the leaf lobes and of the gemmae, as regards pigment formation, is one of the notable features of this complex*, isolating it from all others in the subgenus *Lophozia*. Both also agree in the form, size, and number of the oil-bodies, as well as in gemma size. Therefore, leaf shape, and cell size remain the only effective, obvious means of distinguishing the two taxa. These distinctions, and their values, are clear from plants of *Schuster 35626* (Cape Belknap, Ellesmere Island), where two plants, growing within 1–2 mm of each other, show the following distinctions:

| | *L. pellucida* (typical) | *L. pellucida* var. *minor* |
|---|---|---|
| Marginal cells | (32) 35–40 (45) $\mu$ | 23–28 (30) $\mu$ |
| Subapical cells | 32–38 × 38–46 $\mu$ | 23–28 (30) × 23–30 (34) $\mu$ |
| Median cells | (30) 32–36 × 35–48 $\mu$ | 23–30 × 23–34 (38) $\mu$ |
| Basal cells | 35–40 × 40–48 $\mu$ | 28–32 × 32–38 $\mu$ |

It must be kept in mind that both plants grew under totally identical conditions, and under relatively exposed and xeric conditions (much more so than those characterizing the type locality of *L. pellucida*). The distinctions between the two taxa may be associated with polyploidy. They therefore appear to be identical in nature, and in the mechanism, as those distinguishing *Plagiochila asplenioides* and *arctica* (considered specific) and *Tritomaria quinquedentata* and *T. quinquedentata* var. *grandiretis* (considered merely varietal). Obviously there is a lack of consistency in treatment.[3]

The status of the difficult, smaller-celled phase remains to be decided on the basis of culture work and chromosome counts. The marked differences typically present suggest we deal, at least, with a distinct variety:

*4. Lophozia pellucida* var. *minor* var. n.

Similis regulari *L. pellucidae*, sed plerumque pallidior, lobi folii fuscentes. Folia 1.08–1.48 latiores quam longa. Cellulae minores: marginales 23–27 $\mu$; medianae (23) 25–30 × (27) 30–36 $\mu$. Gemmae 28 × 30 ad 32 × 30–36 $\mu$.

[3]It seems relevant to point out that in the *L. pellucida* complex the two phases have essentially the same number of oil-bodies per cell. In the two analogous cases cited in *Plagiochila* and *Tritomaria*, the putative polyploids average about twice as many oil-bodies per cell as in the haploid stem form.

Typus: Hilgard Bay, NE. Ellesmere Island (*Schuster 35237*).

Plants as in *L. ventricosa* but commonly a paler, more pellucid green, often suffused by a clear, yellowish- or chestnut-brown coloration; without reddish pigmentation of the stem or postical leaf bases, the *leaf lobes* (*and sometimes entire distal halves of leaves*) *becoming clear, yellowish brown* in sun forms. Stem rather soft-textured, ca. 380–450 $\mu$ in diameter; dorsal and lateral cortical cells ca. (18) 20–24 × 65–90 (100–120) $\mu$, averaging 3–5× as long as wide, rectangulate, and quite thin-walled. Leaves broadly ovate to transversely oblong, from 930–1050 $\mu$ wide × 650–970 $\mu$ long to 1100–1175 $\mu$ wide × 750–875 $\mu$ long up to 1400 $\mu$ wide × 1050 $\mu$ long (*ca. 1.08–1.48× as wide as long*), nearly flat to weakly concave, never canaliculate-concave, one-quarter- to one-third- (two-fifths-) bilobed, the sinus obtuse to rectangulate, occasionally rounded. Cells with relatively small trigones (not distinctly bulging), *smaller*; the marginal 23–27 $\mu$ (locally 30–35 $\mu$ long); the subapical 23–25 (30) × 27–32 (35) $\mu$; the median (23) 25–30 × (27) 30–38 $\mu$. Cells with (5–8) 9–15 oil-bodies per median cell, formed of small, slightly protuberant spherules, spherical and 4–5 $\mu$ in diameter to ovoid or ellipsoid and 4 × 4.5 to 5 × 6.5–8 $\mu$, averaging slightly larger than the chloroplasts (which are 4–5 $\mu$ long). *Gemmae of the same color as the leaf lobes* (or sometimes barely more intensely pigmented), their walls colorless (then gemmae masses pale green) to *yellowish brown* (then masses appearing yellowish brown), two-celled, ca. 28 × 30 to 32 × 30–36 $\mu$ when mature.

*Type*

Damp, weakly basic, steep slope, S. end of Hilgard Bay, 82°26′ N., 63° 25′ W., NE. Ellesmere Island (*Schuster 35237*).

*Distribution*

Known only from northern Ellesmere Island. In addition to the type, the following paratypes:

ELLESMERE ISLAND: South end of Hilgard Bay, ca. 82°26′ N., 63°25′ W., NE. coast (*Schuster 35235a, p.p., 35251*); low slope, edge of polar ice cap, 1.5–2 miles W. of Cape Belknap, 82°32′ N., 62°20′ W. (*Schuster 35638, 35632, 35634c, 35638a, 35823, 35820, 35824*).

*Ecology*

At least in the high Arctic of North America this variety appears to be confined to cold, snow-fed slopes, usually under weakly to distinctly calcareous conditions, associated with tolerant species or calciphytes (*Lophozia gillmani, L. muelleri, L. badensis, L. quadriloba, Cryptocolea imbricata, Aneura pinguis, Cephalloziella arctica, Arnellia fennica, Blepharostoma trichophyllum brevirete,* and *Solenostoma schiffneri*).

*Differentiation*

A systematically somewhat baffling taxon. Superficially at times suggesting a derivative of *L. ventricosa*, with which it agrees in the form, size, and number of oil-bodies, in the leaf shape and depth and form of sinus. However, *L. pellucida minor* differs from *L. ventricosa* in that the gemmae parallel closely the leaf cells as regards pigmentation of their walls, i.e., the walls of the

gemmae are colorless when the leaf cells have colorless walls, and are brownish-pigmented when the cell walls of the leaf lobes bear a similar pigment.[4]

The pigmentation pattern, form, size, and number of the oil-bodies, and gemmae size agree closely with that of *L. pellucida*, a common species in the same area, as do the dimensions (and proportions) of the cortical cells of the stem. However, *L. pellucida* has more polymorphous leaves, some of which are markedly broader, on the robust phases bearing a much more shallow, crescentic sinus (and on weaker phases commonly with rounded leaf lobes). In spite of the marked differences in cell size, it remains an open question whether *L. pellucida* and var. *minor* are fully distinct. A situation parallel to that of the *Cephalozia ambigua–bicuspidata–lammersiana* complex may be at hand.

5. *Lophozia* (*Lophozia*) *groenlandica* (Nees *in* G. L. & N.) Macoun
   *Jungermannia groenlandica* Nees *in* G. L. & N., Syn. Hep., 114,1844; Jensen, Oefv. Kgl. Vetensk.-Akad. Förh., No. 6:800, Figs. 1–15, 1900.
   *Sphenolobus groenlandicus* Steph., Spec. Hep. 2:164, 1902; K. Müller, Rabenh. Krypt.-Fl. 6(1):617, 1910, and 6(2):762, 1916.
   *Lophozia groenlandica* Macoun, Cat. Canadian Plants 7:19, 1902.
   *Lophozia murmanica* Kaal. *in* Bryhn, Rept. 2nd Norwegian Arctic Exped. in the "Fram" 1898–1902, 2(11):34, 1906 (*teste* Müller, 1916).
   *Jungermannia murmanica* Arnell, Arkif. f. Bot. 15(5):97, 1917.
   *Lophozia wenzelii*, in part, of authors, including Schuster, Amer. Midl. Nat. 49(2):367, Pl. 20, Figs. 4–10, 1953; Müller, Rabenh. Krypt.-Fl. ed. 3, 6(1):655, 1954.

Plants usually in spongy to compact patches, often densely caespitose and erect or suberect in growth, pure green but often with *leaf lobes somewhat yellowish brown, with ventral side of stem brownish purple or reddish brown*; shoots 1.3–1.8 (2) mm wide × 1–3 (4) cm long, sparingly terminally, furcately branched, rather flexuous. Stems ca. (340) 425–480 μ in diameter, the dorsal and lateral cortical cells ca. 23–27 (29) μ wide × 42–60 (80) μ long, hardly thick-walled; medulla very strongly dorsiventrally differentiated, the four to six dorsal layers colorless and pellucid, large-celled (the cells ca. 25–33 μ in diameter), the 10–15 ventral tiers very small-celled (cells ca. 10–15, rarely to 20 μ in diameter); ventral medullary cells somewhat dull rose to reddish-tinged, and with age strongly mycorrhizal. Rhizoids often rose-tinged near base. Leaves *somewhat to strongly antically secund*, slightly to quite distinctly imbricate, *quite obliquely inserted* (*and oriented*), and slightly decurrent antically, *rotund-quadrate*, averaging (on mature shoots) from (930–1050) 1150–1200 μ wide × (920–980) 1000–1100 μ long, occasionally to 1300–1350 μ wide × 1200–1225 μ long or 1550 μ wide × 1300 μ long (*averaging from 1.05–1.15* (*1.2 × as wide as long*, but on small-leaved, juvenile shoots sometimes slightly less broad), *widest commonly above the middle, quite strongly concave* (although

[4]Although one might, at first, wish to dispose of var. *minor* as a larger-celled, Arctic derivative of *L. ventricosa*, this appears impossible to maintain. Both *L. pellucida* (typical) and *L. ventricosa* (typical) occur on "The Dean" in NE. Ellesmere Island. The former is a plant of low, calcareous, wet slopes; the latter of high, non-calcareous, peat-covered scree slopes. The strongly calciphytic restriction of *L. pellucida*, vs. distinctly oxylophytic restriction of *L. ventricosa*, suggests clearly that the calciphytic var. *minor* belongs with *L. pellucida*, rather than with *L. ventricosa*.

scarcely hemispherical, *never canaliculate*), *and with incurved lobes, very shallowly bilobed*; *sinus extending for 0.5–0.15* (rarely to 0.25) *the leaf length, usually crescentic* and very broad, *the lobes broadly obtuse*; leaf margins usually moderately to strongly arched, the antical often less so. Cells distinctly collenchymatous, those of the leaf tips and margins with their walls commonly yellowish to yellowish brown, ca. 20–24 × 21–27 μ, the median cells 23–26 μ wide × 24–32 (33) μ long; cuticle smooth; oil-bodies (in all but exceptional individuals) *biconcentric*, with a centric or excentric, interior pale bluish appearing, homogeneous oil-spherule, surrounded by a granular peripheral mantle of minute spherules, *usually* (*8–10*) *12–15* (*17–18*) *per median and submedian cell*, often largely spherical and 4.5–6 μ, but frequently in large part ovoid to irregularly ellipsoid and up to 4.5–5 × 7–10 μ. *Gemmae pale greenish*, in relatively small quantities, from apices of uppermost leaves, one- to two-celled and polygonal, or quadrate to stellate, from 18–20 × 21–25 μ up to (occasional maximum) 23–24 × 28–32 μ, their walls slightly, their angles prominently, thickened.

Dioecious. Female bracts usually dimorphic: one bilobed (ca. 1500–1550 μ wide × 1300 μ long), the other three- to four-lobed (ca. 1400 μ wide × 1200 μ long), erect and somewhat sheathing perianth at base, but with spreading lobes; bracteole usually distinct as a lanceolate lobe, up to 500 μ wide × 1000–1200 μ long, united for up to 0.5–0.6 its length with one of the bracts; margins of bracts and bracteole entire. Perianth at mouth *with remote one- to two-* (*three-*) *celled teeth*, 35–55 μ long; end cell of teeth narrowly triangular, ca. 25–35 μ long × 13–15 μ wide.

*Type*

Greenland.

*Distribution*

This poorly known and long-forgotten species appears to be essentially arctic-alpine in distribution, with a disjunct extension to the Great Lakes region. It occurs northward virtually to the northernmost periphery of the northern hemisphere (82°26′ N. lat.). The range of this species is probably much confused in the literature with that of *L. wenzelii* and *L. ventricosa*; the following reports are the only certain ones known to the author:

GREENLAND: Narsak Fiord, W. coast (*Vahl, Breutel*; type); South coast (*Böck*); Hurry Inlet, E. coast (12, p. 800; ♂ plant.)

ELLESMERE ISLAND: "The Dean," 5 miles S. of Alert, 82°26′ N., 62°05′ W., NE. Ellesmere (*Schuster 35393*; northernmost station for species!); King Oscar Land and Ellesmere Land (4, as *L. murmanica*).

MICHIGAN: Pictured Rocks, Alger Co. (*Schuster 39294, p.p.*, among *Lophozia incisa, Gymnocolea inflata, Cephalozia bicuspidata*, on sunny, damp sandstone ledges).

MINNESOTA: Porcupine Island, Susie Islands, Lake Superior shore near Grand Portage, Cook Co. (*Schuster 19409*).

Also reported (17, p. 617) from Spitsbergen, *teste* Bryhn, and (17, p. 763) from Lapland (near Litsa, type of *L. murmanica* Kaal.), and from alpine situations in central Europe (Feldberg, Baden, *Müller*; Monte Coglians, Friaul, 2100 m, *Kern*). The latter two reports need verification.

*Ecology*

Associated with ledges and cliffs, and exposed rocks, either on north- or northwest-facing cliffs, in direct sun to permanent shade (Lake Superior) or on exposed peaty soil over weathered mountain slopes (Ellesmere Island). Associated in the former case with *Dicranum*, and in the latter case with *Rhacomitrium lanuginosum*, *Tritomaria quinquedentata*, *Eremonotus minutus*, *Lophozia alpestris major*, *Cephaloziella arctica*. In Greenland it is reported as occurring with *Anthelia julacea*; in Michigan on cold lake bluffs, over sandstone, with members of the oxylophyte *Gymnocolea – Cephalozia bicuspidata* Associule. In the far north on steep slopes, over non-calcareous rocks, in areas where snow lies late into the spring (to June 15–30), and where the moss-tundra is kept damp by melting snowbanks during the remainder of the season.

*Differentiation*

The pale greenish gemmae and very broad leaves, normally bilobed (rarely sporadically trilobed) by a shallow and crescentic sinus suggest the commoner *L. wenzelii* and phases of *L. ventricosa*. When living material is at hand, confusion with these two is hardly possible, due to the biconcentric oil-bodies, very similar to those of *L. silvicola*. However, the much broader leaves, of mature plants, with the leaves quite antically secund, particularly when the plants are crowded, concave, and with incurved lobes, and the usually yellowish-brown pigmentation of the leaf lobes, give the plants a very different facies from *L. silvicola*. Furthermore, the pigmentation pattern of these two species appears to differ considerably, *L. silvicola* tending to have (in sun forms) a bright carmine or purplish pigmentation of the leaf bases; such pigmentation appears to be consistently absent in *L. groenlandica*, except rarely at the apices of immature perianths.

The situation with respect to *L. wenzelii* and *L. ventricosa* is very different, and it is very questionable whether *L. groenlandica* will always prove distinguishable except on the basis of living plants.[5] *L. groenlandica* closely approaches *L. wenzelii* in the rotund-quadrate leaves and very shallow sinus, as well as in the tendency for the leaves to be quite strongly concave. There are, however, distinct differences in facies: *L. groenlandica* has considerably more obliquely inserted leaves, and the leaves are usually more antically secund. To this must be added the considerable difference in the oil-bodies, those of *L. groenlandica* being biconcentric and very similar to those of *L. silvicola*, whereas *L. wenzelii* agrees with *L. ventricosa* in the possession of oil-bodies formed throughout of minute spherules.

The oil-bodies, in *L. groenlandica*, are more polymorphous than in *L. silvicola*. In some cases (*inter alia*, in the Lake Superior plants) the oil-bodies are almost all spherical, and the larger, included oil-spherule is nearly centric in most instances (21, Pl. 20, Fig. 8). In the Ellesmere Island plants, however,

[5]The author's experience with the species perhaps bears repeating: when first "exposed" to this difficult species, on the basis of Lake Superior material, it was confused with *L. wenzelii*. When, 4 years later, it was seen again, a second time, in Ellesmere Island, the specific identity of the Minnesota and Ellesmere Island plants was at once suspected: the identity in facies was quite marked. Probably, after once being correctly recognized, the species can be identified even on the basis of dead material.

many oil-bodies, if not most, are ovoid or irregularly ellipsoidal in shape, although perfectly spherical ones are frequent, and the included oil-globule is more often excentric than centric. The oil bodies may average somewhat less numerous than in *L. silvicola* (where they are usually 15–22, occasionally 25 per median cell). In most cases there are 12–15 oil-bodies in the median cells, although not uncommonly there are only 8–10; very exceptionally there may be as many as 17 or 18 oil-bodies. In oil-body number, therefore, *L. groenlandica* closely agrees with *L. ventricosa* and *L. wenzelii*.

The writer has studied *L. groenlandica* intermittently for over 10 years. At first it appeared impossible to keep it clearly apart from *L. wenzelii*, and material of it was reported (21) as *L. wenzelii*, The species is also generally considered in the literature (9, 18) as a simple synonym of *L. wenzelii*. Furthermore the Minnesota plants were determined by the late Dr. Müller as *L. wenzelii*. It must be admitted, as has been pointed out in Schuster (21) that Dr. H. Buch did not consider the Minnesota material to be *L. wenzelii*. He considered it to be a large-celled phase of *L. alpestris* (*L. alpestris major* C. Jensen), but admitted that if it differed from *L. alpestris* not only in cell size, but also in the possession of *L. silvicola*-like oil-bodies, it "könnte dann vielleicht als Art behandelt werden. Dann muss sie aber allem Anschein nach *L. grönlandica* (Nees) bennant werden."

With the study of living plants of this taxon (summer 1955, in northern Ellesmere Island; summer 1957, northern Michigan), the writer found that there was again a correlation between a broad, *L. wenzelii*-like leaf with a very shallow sinus, pale green gemmae, and *L. silvicola*-like oil-bodies, *exactly as in the Minnesota material*. Therefore, it hardly appears possible to retain these plants as a synonym of *L. wenzelii*.[6] In point of fact, it was emphasized, in connection with the Minnesota material (21, p. 369) that "(1) either a discrete species is at hand, or (2) *L. wenzelii* is at hand. Until study of living *L. wenzelii* will show that it has different oil-bodies than those figured here, treatment of the present collection under this name appears wisest." Since then, living material of *L. wenzelii* has been studied (from northern Sweden, through the courtesy of Dr. S. Arnell; and from Mt. Katahdin, Maine), and has shown to have consistently *L. ventricosa*-like oil-bodies. No other alternative, therefore, exists but to consider *L. groenlandica* as distinct.

The application of the name *L. groenlandica* to this taxon is perhaps not above criticism. Müller (17, Vol. 1, p. 617) discussed this species briefly and came to the conclusion that one "can be in doubt, in this species, whether it is to be placed in *Lophozia* or *Sphenolobus*" but later (17, Vol. 2, p. 762) concluded that "*Jg. groenlandica*" belongs in the "Formenkreis" of *L. wenzelii*. Furthermore, *Lophozia murmanica* Kaal. is considered to be "very close" to *L. groenlandica*, and is placed as a synonym of this species, which in turn is regarded as identical with *L. wenzelii*. This conclusion has been generally accepted since (9, 21, 18), and *L. groenlandica* is not listed in the American

[6]It should be noted, in passing, that *L. alpestris* var. *major* is abundant in northern Ellesmere Island. Indeed, a few plants were found admixed with the *L. groenlandica*. It is always readily separable by (*a*) reddish gemmae; (*b*) much smaller trigones; (*c*) flatter leaves with usually deeper, angular sinus; (*d*) non-biconcentric oil-bodies. The attempt by Buch to equate *L. alpestris* var. *major* with *L. groenlandica* is clearly impossible to follow.

literature of the last 40 years. The name *L. groenlandica* is revived for the present taxon for two reasons (*a*) Nees's *Jungermannia groenlandica* (as regards description) is inseparable from the present plants; (*b*) the application of Nees's species name to the present plants effectively removes the necessity for proposal of a new species name. The identity of the type of *J. groenlandica* with the present plants will never be susceptible to unquestionable and final proof, since the oil-body characteristics of the type of *J. groenlandica* cannot be determined. An additional element of uncertainty is introduced by the fact that Nees's type shows a high incidence of trilobed leaves, while the material assigned to this species by Jensen (12) and Bryhn (4)—as well as that assigned to it by the writer—has preponderantly to almost uniformly bilobed leaves, only isolated and sporadic leaves being trilobed.

The distinction of *L. groenlandica* from *L. ventricosa* is at times very difficult, unless living plants are available for study. This is clearly illustrated by the Ellesmere Island plants, which occurred among *Eremonotus minutus*, in a small area lying within 2 ft of a patch of *L. ventricosa* (also admixed with *E. minutus*). The plants, in both cases, had concave, antically secund leaves, with somewhat incurved lobes. The xeromorphic phase of *L. ventricosa*, indeed, had much the facies of a small *L. groenlandica*. However, the following distinctions occurred: (*a*) *L. groenlandica* had the ventral side of the stem, and sometimes the adjacent portion of the postical bases of the leaves, as well as the rhizoids rose- to reddish-tinged; in *L. ventricosa* such pigments were lacking, and the stem, with age, becomes brownish from mycorrhizally induced breakdown of the ventral portions of the stem; (*b*) *L. groenlandica* had an average of 12–15, occasionally 17–18, biconcentric oil-bodies per cell; in *L. ventricosa* the somewhat xeromorphic plants which were found had only (5–7) 9–15, rarely 16, oil-bodies per cell, all of which were formed of spherules of uniform size; (*c*) *L. groenlandica* had somewhat broader leaves, occasionally as much as 1.15–1.2× as wide as long, with a generally notably shallower and broader sinus, and thus more obtuse lobes; in *L. ventricosa* the leaves averaged only 0.95–1.1× as wide as long, and many leaves had a more distinct, if obtuse sinus, and subacute lobes. Although these distinctions are slight, except for that dealing with the oil-bodies, the two taxa are immediately separable under the binocular microscope by the sole difference in color (indicating a different physiological response to identical insolation!), and were recognized as palpably distinct in the field. In both cases the leaf cells, at least of the distal half of the leaf, tend to develop a light golden or yellowish pigmentation, although in *L. ventricosa* a slight tendency for a reddish tinge is locally present on the leaf lobes.

The plants from the Lake Superior shore of Michigan are critical in some respects. When typically developed they bear all the features characteristic of *L. groenlandica*, including the biconcentric oil-bodies; very broad, rotund-quadrate leaves with a broad, shallowly crescentic sinus; a concave leaf form, with the blunt and broad lobes distinctly incurved. Such plants have leaves varying from 1.09–1.33× as broad as long (typical measurements: 1150 × 950 $\mu$; 1200 × 900 $\mu$; 1250 × 1050 $\mu$; 1480 × 1150 $\mu$). However, a single case has been seen of a slender, *L. silvicola*-like shoot bearing spreading-almost squarrose leaves giving rise to a branch with concave, broad, *L. groen*,

*landica*-like leaves. The weak parent axis had leaves only 830–860 × 880–890 μ to 900 × 820 μ to 1025 × 1000 μ long (width: 0.93–1.1× the length), although the sinus was shallow and crescentic; the strong branch had the leaves from 960 μ × 780 μ long to 1100 μ × 800 μ long (1.26–1.37× as wide as long). It is therefore evident that under presumably "difficult" conditions, during certain seasons, *L. silvicola*-like extremes may be produced that possibly cannot be separated from *L. silvicola* except by culture experiments.

## Acknowledgments

As usual, my wife, Olga M. Schuster, has helped both in editing and typing the manuscript and in preparing the specimens for the herbarium. Dr. Doris Raymond kindly prepared the Latin diagnoses. The materials reported on here were principally collected while the writer was at Alert, Ellesmere Island, in the summer of 1955. I am indebted to the Arctic Weather Stations, operated jointly by Canada and the United States, and to the United States Weather Bureau, in making it possible for me to reside and do field work in the area. Dr. Sigfrid Arnell has given me the benefit of his opinion with respect to *Lophozia pellucida* and has kindly supplied the Swedish specimen of this species cited.

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# NOTES

## THE PRODUCTION OF THE ASCOGENOUS STAGE OF DIDYMELLA APPLANATA (NIESSL) SACC.[1]

J. B. JULIEN AND A. T. BOLTON

### Introduction

A survey of the spur blight disease of raspberry made in Ontario and Quebec from 1956 to 1959 showed that the average raspberry plantation suffers an estimated 25% crop reduction from spur blight due to defoliation and predisposition to winter injury.

Koch (4) studied the growth of *D. applanata* in culture but did not succeed in obtaining all stages in the life cycle of the pathogen on artificial media. This inability of the fungus to reproduce the perfect stage in vitro has been an important factor in preventing genetic study of this fungus. One must know the details of its reproduction to study the transmission of virulence and the existence of pathological races, and finally to integrate this knowledge efficiently and economically with a breeding program to produce raspberry varieties resistant to *D. applanata*.

### Materials and Methods

Cultures of the fungus were obtained from tissue isolates, and from ascospores discharged directly onto an agar medium. These cultures were kept alive by frequent transfers on P.D.A.[2] Suspensions consisting of a mixture of conidia and hyphae were used to seed the following media:

(1) pieces of raspberry cane sterilized in a propylene oxide atmosphere and half-buried in P.D.A.;
(2) P.D.A. prepared by using equal parts of water and a decoction made by steaming 25 g of dried raspberry cane bark in ½ liter of water;
(3) Hawker's (2) basal medium to which was added one of the carbohydrates dextrose (0.5), starch (2.0), arabinose (1.0), or sucrose (5.0) and neither, one, or both of the vitamins thiamine and biotine (4 μg/100 ml of medium);
(4) water agar (1.5%) to which was added 1.0% of one of the carbohydrates dextrose, cellulose, or chitin;
(5) checks: P.D.A. and water agar (1.5%).

The seeded Petri dishes were placed in an incubator and the temperature controlled (so that it corresponded *grosso modo* to the variation occurring in nature from the late summer infection to the spring discharge of the ascospores)

[1]Contribution No. 48, Genetics and Plant Breeding Research Institute.
[2]Potato-dextrose agar (1.5% dextrose).

as follows: 1 week at 20° C, 2 weeks at 15° C, 1 week at 10° C, 1 week at 5° C, 1 week at 0° C, 1 week at 5° C, 1 week at 10° C, 1 to 3 weeks at 15° C.

## Experimental Results

Growth and reproduction were observed on the above media. The fungus produced numerous pycnidia but no perithecia on P.D.A., on pieces of sterilized cane in P.D.A., or on the raspberry bark decoction – P.D.A. mixture. Several of the carbohydrates and vitamin mixtures promoted pycnidial production, but none favored the production of perithecia.

A reduction in food supply has been shown to initiate formation of fruiting bodies in many fungi (1, 3, 5). In the present experiment the media consisting of 1.5% agar in distilled water without carbohydrates, or with cellulose or chitin, produced perithecia, demonstrating for the first time that *D. applanata* can complete its life cycle in artificial media. More perithecia were produced on the above media at pH 5.2 than at pH 6.2, except on the media containing cellulose, on which the number of perithecia produced was practically the same at both pH's.

Perithecia in culture could be differentiated from the pycnidia by their spherical appearance and the presence of mature asci. Pycnidia are gregarious or solitary and their size, color, and wall toughness vary with the medium. They ooze conidia soon after formation.

## Conclusion

These experiments are exploratory and, consequently, do not permit detailed analysis of the results or make generalization feasible. However, one may conclude that a reduction of nutrient favors the production of perithecia of *D. applanata*.

Further work on the effect of variation in temperature upon the production of fruiting bodies is in progress.

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# AN OCCURRENCE OF OOSPORES IN CANADIAN CULTURES OF PHYTOPHTHORA INFESTANS (MONT.) DE BARY[1]

K. M. Graham and Fay Wright

Observations of the occurrence of oospores without antheridia in pure cultures of *Phytophthora infestans* have become rather commonplace in the course of studies of the organism. The appearance of oospores with amphigynous antheridia has been rare with the exception of the report by Smoot *et al.* (7) and Gallegly and Galindo (4) that they were produced in profusion by the pairing of isolates of the $A_1$ and $A_2$ compatibility groups. Other documented cases were noted by Clinton (2) in 1911, Pethybridge and Murphy (5) in 1913, and Berg (1) in 1926. Gallegly (personal communication) concluded that their occasional appearance within isolates belonging to the $A_1$ group, within which all of our Canadian isolates fall, was of parthenogenetic origin. It is also possible that they are the result of an infrequent mutation from compatibility group $A_1$ to $A_2$, from $A_1$ to homothallism, or an expression of relative sexuality among $A_1$ group members with varying levels of "maleness" and "femaleness".

A search for oospores in Canadian isolates was initiated in 1958, by a procedure of selective pairing. Thirteen isolates from our stock culture collection were paired in all possible combinations on V-8 juice agar made up according to Smoot *et al.* (7). One pairing produced four oospores (Fig. 3) in the zone between the colonies. One of the two isolates, No. 167, was paired with 90 other isolates of the culture collection. On the basis of the frequency of occasional production of oospores, isolate No. 121 was chosen for further pairings with No. 167.

A basic assumption was made that a genetic change may have occurred in one or more of the nuclei in the hyphae at the sites of origin of the oospores in the pairing No. 167 × No. 121. In order to conserve any heterokaryon bearing nuclei with factors for the $A_2$ compatibility type or for homothallism, nine of the oospores with their adhering mycelium were cut out of the agar with a "biscuit cutter" and cultures from these were established on lima bean agar. The nine isolates were then paired with each other in all possible combinations. Five pairings produced oospores at the rate of one or two per plate. Eight of these were selected by the biscuit-cutter method and the resulting cultures were paired with each other. In this generation, a total of 455 were produced, a large number of which were abortive (Figs. 1 and 2), but 83 appeared to be authentic oospores with amphigynous antheridia (Figs. 4 and 5). They compared favorably with those produced by matings of the $A_1$ and $A_2$ types. An example of one of these is reproduced in Fig. 6 from Gallegly and Galindo (4).

Throughout the succession of selective pairings the oospores were formed on one colony or the other; there was no evidence to indicate that they had been formed after contact of hyphae from the two partners of any given

[1]Contribution No. 58, CDA Research Station, Fredericton, New Brunswick.

pairing. Indeed, those formed in the last set of pairings were not confined to the zone between the colonies but appeared frequently around the point of inoculation of the medium. A reasonable explanation would be that they were formed within unfertilized oogonia stimulated in one isolate as a result of a diffusible hormone produced by the other. Their formation might be interpreted as part of the phenomenon of relative sexuality demonstrated in *Achlya* by Raper (6) and found to be operating in isolates of the $A_1$ and $A_2$ compatibility types of *P. infestans* by Galindo and Gallegly (3). Concomitant with the production of oogonia, however, a change occurred which apparently could be perpetuated through subcultures made from the hyphae in their immediate vicinity and which culminated in the production of oogonia with antheridia. The question of whether this change involved a series of step mutations from $A_1$ to $A_2$ followed by an authentic pairing or a mutation from $A_1$ to homothallism must await an exhaustive single-spore analysis of the cultures involved.

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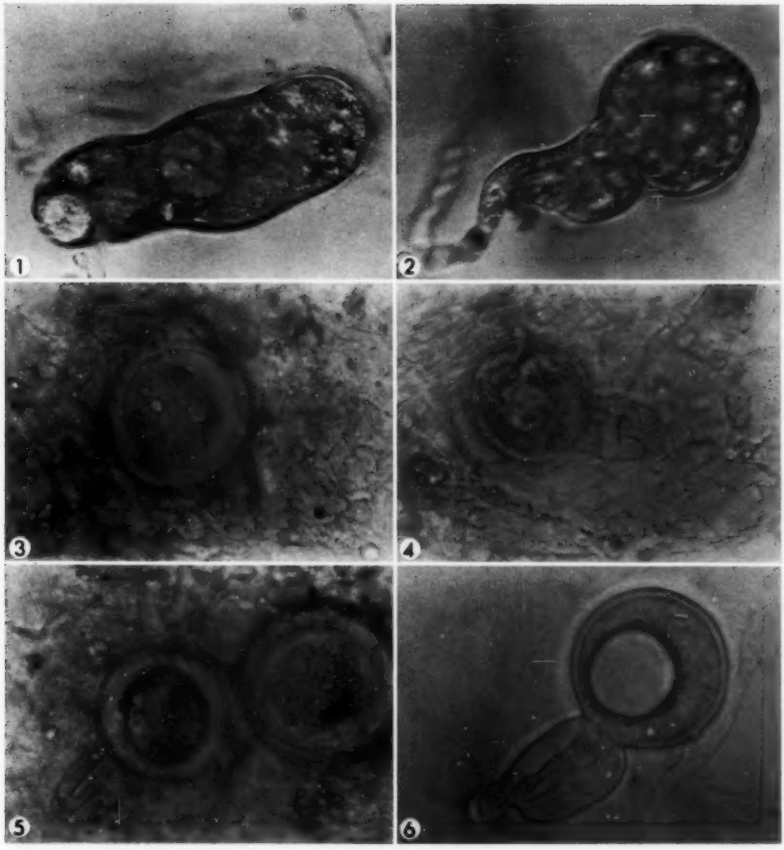
Note: Figs. 1–6 follow.

PLATE I




FIGS. 1 and 2. Bodies believed to be abortive oogonia, produced on V-8 juice agar by the pairing of Canadian isolates Nos. 167 and 121 of *P. infestans* (×900).

FIG. 3. Oospore without amphigynous antheridium, found after pairing Nos. 167 and 121 (×900).

FIG. 4. Immature oospore with amphigynous antheridium (×900).

FIG. 5. Left: oospore with amphigynous antheridium; right: oospore without antheridium (×900).

FIG. 6. Oospore with amphigynous antheridium produced by pairing isolates of the $A_1$ and $A_2$ compatibility (reproduced by permission of the authors from Gallegly and Galindo (4)) (×900).

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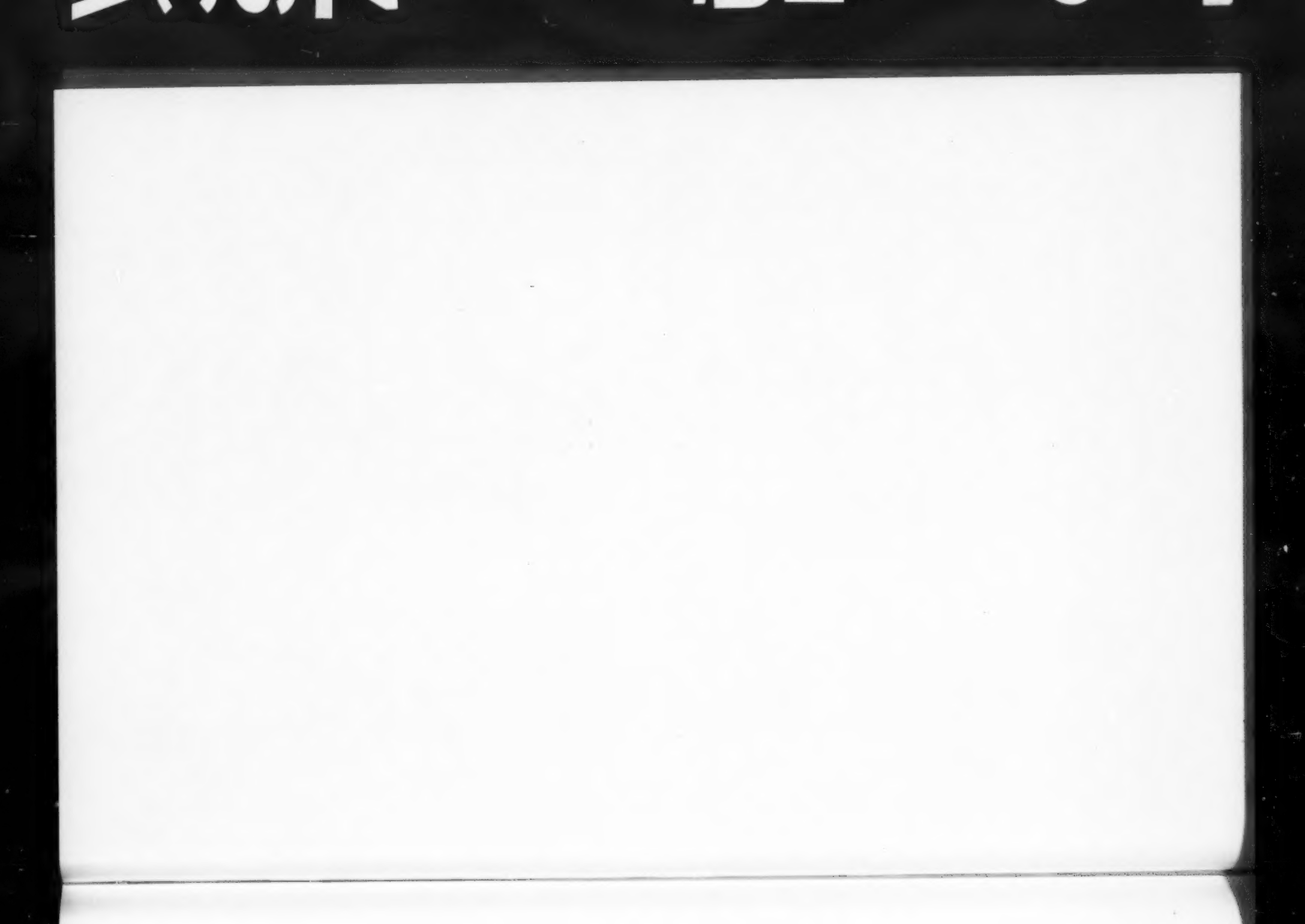

# NOTES TO CONTRIBUTORS

## *Canadian Journal of Botany*

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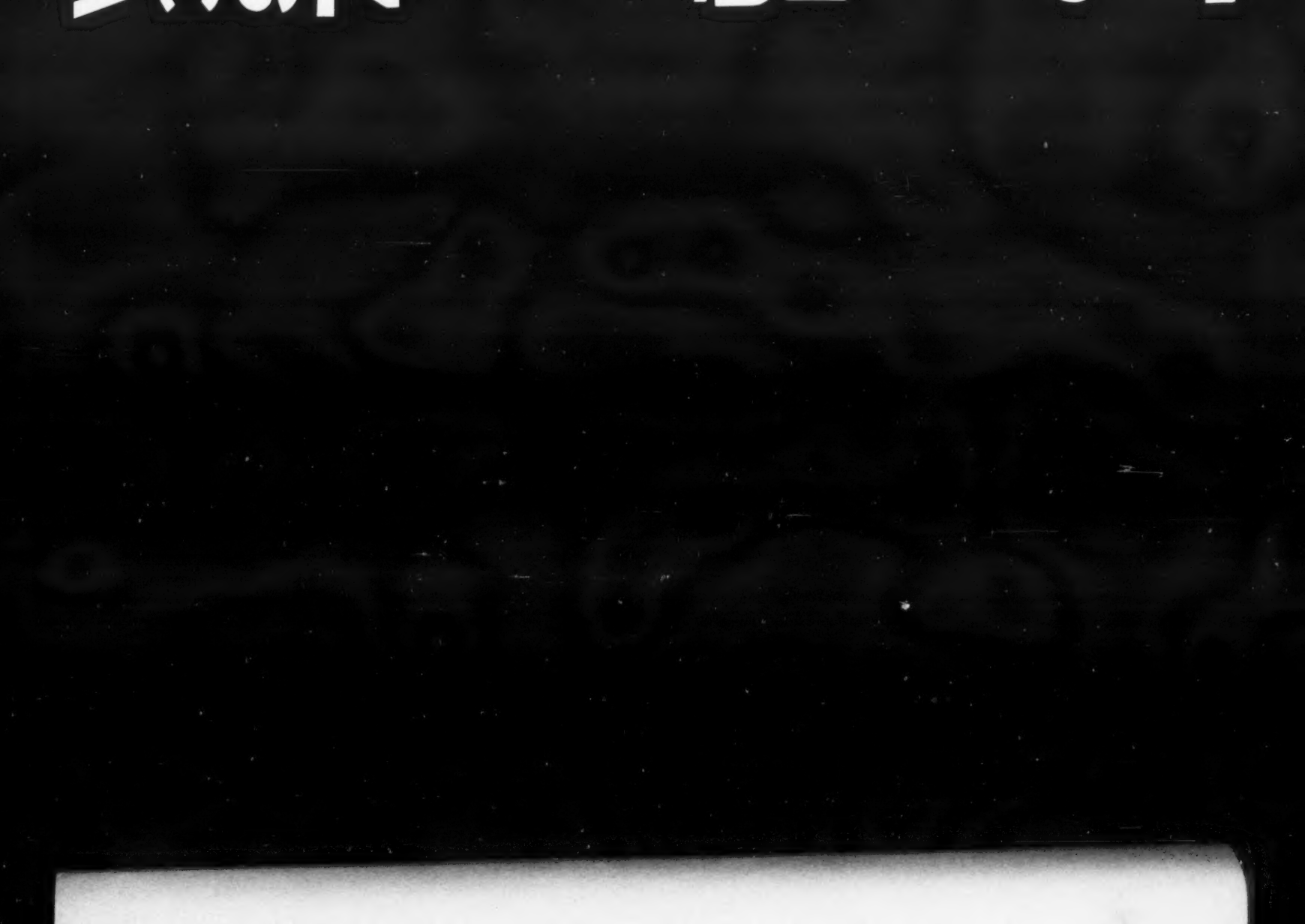
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